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MELBOURNE

SOME EFFECTS OF TEMPERATURE ON THE DIURNAL PERIODICITY OF ADULT EMERGENCE IN *TRICHOPODA PENNIPES* (DIPTERA: TACHINIDAE)

By F. Wilson* and G. J. Snowball*

[*Manuscript received September* 30, 1958]

Summary

There is a marked diurnal periodicity of adult emergence in *Trichopoda pennipes*. This periodicity is greatly modified by temperature change, and adults can be induced to emerge at any time by providing a temperature-increase stimulus. Adults sometimes emerge in response to very small increases of temperature.

I. Introduction

As part of a programme for the biological control of *Nezara viridula smaragdula* (F.) (Hemiptera: Pentatomidae), puparia of the parasite *Trichopoda pennipes* (F.) were imported into Australia from Florida and the British West Indies in 1952 and 1953. Both the typical form and the colour variety *pilipes* (F.) were present in the consignments. Adults of *T. pennipes* emerge predominantly between sunrise and midday (Drake 1920; Beard 1940; O'Connor 1950), and observations on the imported puparia provided information on the effect of temperature on this diurnal periodicity of emergence.

II. Methods

The puparia were kept at a relative humidity of, or close to, 100 per cent., and at temperatures which fluctuated in different ways. Observations were made at rather short intervals on the temperatures occurring and the numbers of adults emerging.

In one series of observations, series I, 450 exposed puparia were kept for emergence in a room in which the temperature fluctuations were similar to outdoor air temperatures, except on the eighth day of emergence when the temperature was increased by electric heating from about 5 a.m. until noon. On each day, the observations began about 4 a.m. and continued at intervals of usually 5–15 min for 16 or more hours.

In another series of observations, series II, puparia were randomly separated into two groups of 1000, X and Y, which were kept for emergence in separate, dark, insulated chambers. The temperature of the chamber containing group X was allowed to fluctuate without artificial modification: the chamber containing group Y was, at different times, heated electrically or cooled by the insertion of ice blocks. The observations were made from 1.30 a.m. to 4.30 p.m. each day, at intervals which varied from a few minutes to one hour.

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In both these series of observations, the numbers of flies emerging outside the hours of observation were negligible.

In other observations, series III, puparia on five occasions were subjected to artificial temperature increases for varying periods between 5 p.m. and 12 p.m., when in nature the temperature is characteristically declining. Fly emergence was recorded.

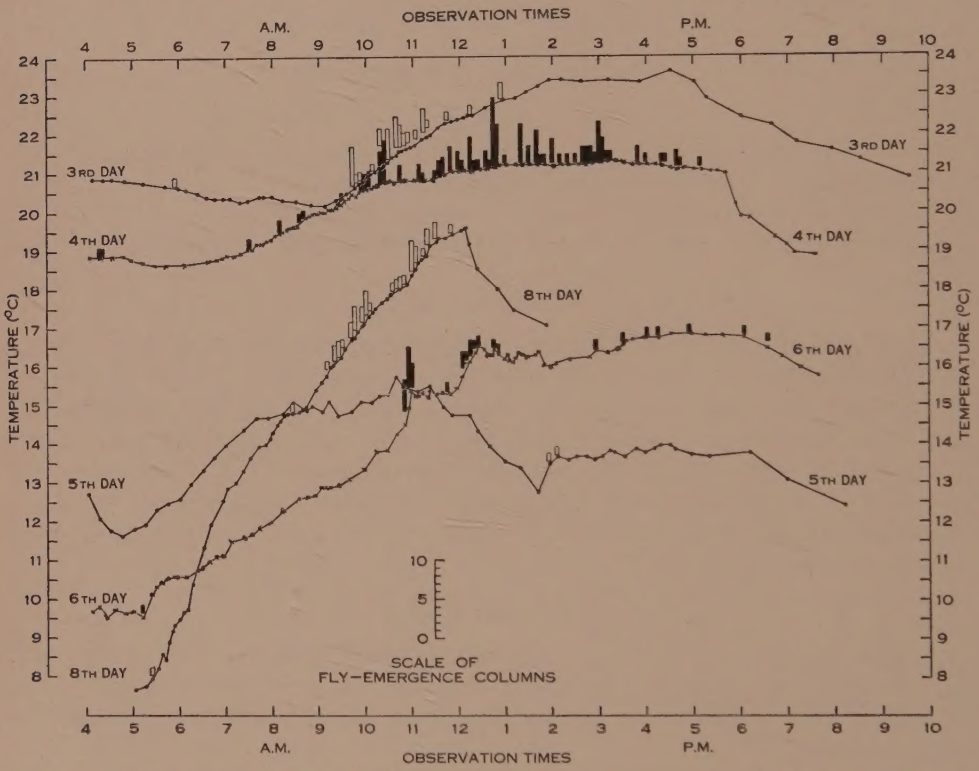

Fig. 1.—Adult emergence of *T. pennipes* (columns) and ambient temperatures (continuous lines) in series I. Temperatures not artificially modified except on 8th day. Emergence columns are shown in outline (third, fifth, and eighth days) and in solid block (fourth and sixth days): temperature readings are shown as dots (third, fifth, and eighth days) and as crosses (fourth and sixth days).

III. Results and Conclusions

(*a*) *Series I*

Figure 1 shows data from series I for the third, fourth, fifth, sixth, and eighth days of the 11 days on which emergence occurred, these five days being selected to illustrate the pattern of emergence associated with markedly different temperature curves.

In Figure I, adult emergence conforms to a general pattern: it is almost confined to the hours between 8 a.m. and 6 p.m., and to temperatures above 15°C. Within these approximate limits, there is much variation in the hours of the day

during which emergence takes place. On the fourth day, when the temperature curve was relatively flat, emergence continued fairly steadily throughout the day until about 5 p.m. On days with a steeply rising morning temperature (third and eighth days) emergence terminated about noon or 1 p.m. On the sixth day, when the temperature rose rapidly throughout the morning from an initially low level, no emergence occured until about 11 a.m. and a temperature of about 15°C had been reached. On the fifth day, with a persistently low temperature, extremely few adults emerged.

It is evident that, with adequately high temperatures, adults emerge during many daylight hours if the temperature is relatively stable, and emergence is completed at a relatively early hour if the temperature is steeply rising: with low temperatures, few adults emerge.

Emergence is completed much earlier than on any day in series I if appropriately high and rising temperatures occur. During other observations, made in the summer at Brisbane, emergence for the day was frequently completed by 9 a.m.

(*b*) *Series II*

In Figure 2, the effects of different temperatures during four successive days (on which most of the emergence took place) upon similar groups of puparia are compared. The temperature curves for group X are relatively flat. With a minimum temperature at 8.30 a.m. and a maximum reached or closely approached at 5 p.m., the daily amplitude ranged from 0·6 to 2·1°C, and the fluctuation during the whole period was not appreciably above ±1°C. Under these conditions, there was a marked emergence periodicity of similar form each day: almost all the adults emerged between 5 a.m. and 3 p.m., and there was a marked peak of emergence in the two or three hours of rising temperature after 8.30 a.m.

The period of the day in which emergence principally occurred in series I and in group X of series II (approximately 5 a.m. to 5 p.m.) is referred to below as the "preferred emergence period".

The pattern of emergence in group X is similar to that obtained with "constant" temperatures by Bateman (1955) with *Strumeta tryoni* and by Brett (1955) with *Drosophila melanogaster*, which suggests that *T. pennipes* possesses a similar mechanism for the production of emergence periodicity to that reported for these species, in which periodicity is produced by preconditioning in an earlier stage of the life cycle. Possibly, emergence in group X was also affected by the small temperature fluctuations occurring.

The pattern of emergence in group Y contrasts sharply with that of group X. Artificial temperature increases at night time, when the temperature would otherwise have been falling, induced substantial numbers of adults to emerge on each of the four days at a time when emergence does not normally occur. Emergence caused by a temperature-increase stimulus continued for a while after the cessation of the stimulus. On the fourth day, three successive temperature-increase stimuli were provided, and three distinct groups of adults emerged.

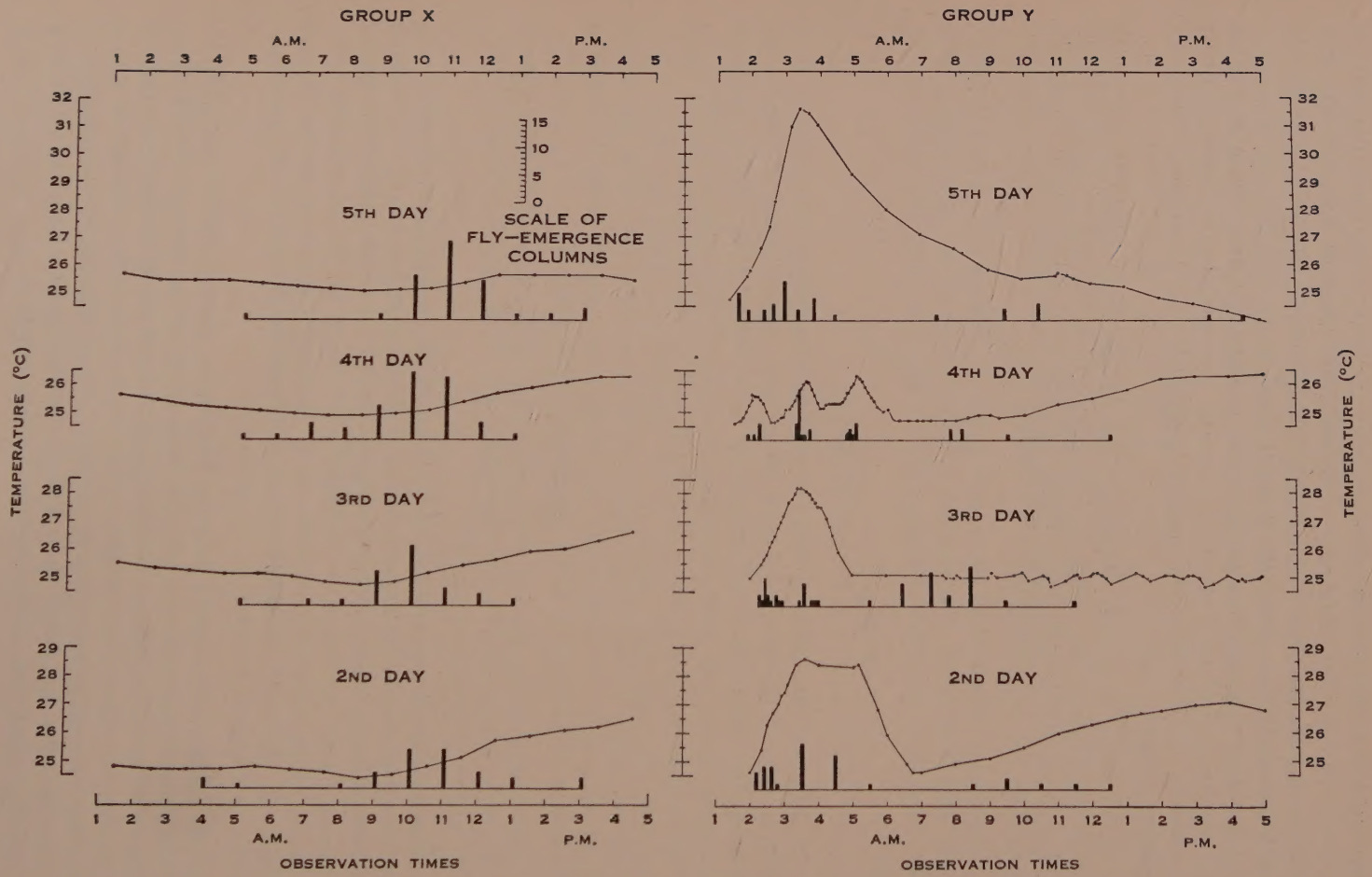

Fig. 2.—Adult emergence of *T. pennipes* (columns) and ambient temperatures (continuous lines) in groups X and Y of series II. Temperatures artificially modified in group Y only.

The forms of the daily temperature curves subsequent to the nightly temperature increases differed considerably on the four days, but on each day, nevertheless, some adults emerged during the preferred emergence period, though somewhat early in this period relative to emergence in group X.

Induced emergence from the puparia of group Y always began very soon after the initiation of the temperature-increase stimulus. In one instance (which was probably not exceptional) the first adult emerged in 15–20 min, the temperature increase at 20 min being 0·5°C. Such insects are clearly very sensitive to temperature increase. However, because of the short time intervals involved, such small temperature increases represent relatively steep temperature gradients. For example (Foley 1945), data on monthly averages of hourly temperatures for Brisbane, the climate of which is similar to that of Florida where the insect is indigenous, show that more than two-thirds of all temperature increases are less than 1·5°C per hour.

(*c*) *Series III*

The following tabulation summarizes the data obtained in the third series of observations in which puparia were exposed on five occasions to artificial temperature increases during the evening hours:

Temp. Increase (°C)	Period (p.m.)	No. of Flies Emerging
2·0	5 –5.30	2
2·6	6 –7	6
6·0	6.30–12	8
6·0	7.30–10	3
8·0	9 –12	6

On each occasion, temperature increase induced emergence. Flies emerged during all hours from 5 p.m. until 1 a.m.

(*d*) *General*

In series II and III, there was great variability in the emergence response to temperature increase. Although some adults emerged very soon after the commencement of heating, others emerged only after the temperature had risen several degrees over a period of one or more hours. The size of the stimulus necessary to induce the emergence of flies is possibly related to the favourableness of the environment for emergence during the preceding day—the more favourable that period for emergence, the greater the stimulus required—or, alternatively, to differences in preconditioning of the insects.

In series II and III, artificial temperature increase at different times in the day caused adults to emerge, and it is clear that emergence can be induced at any hour. The adults which can be induced to emerge at periods of the day in which emergence seldom or never occurs in nature are clearly ready to emerge, merely requiring an appropriate stimulus to do so. That a temperature-increase stimulus, extending over a short time, often caused a relatively large number of adults to emerge shows that puparia containing adults which are ready to emerge accumulate during the periods of non-emergence. In nature, the main emergence from the

accumulated "ready" pupae usually takes place during the earlier part of the preferred emergence period, which usually coincides with a period of temperature increase. When, as is frequently so in nature, a long period of temperature increase in the morning provides ample opportunity for adults to emerge from all the accumulated ready pupae, the continuation of rising temperature in the later part of the preferred emergence period does not cause more adults to emerge.

It is concluded that the observed pattern of emergence in *T. pennipes* is the result of the interaction of two factors: a preferred emergence period, which is probably predetermined, and a response to temperature gradient, temperature increase promoting emergence.

Sex has no influence on the time of day at which adults emerge. During 7 days in series I, 159 males and 160 females emerged, and there was no significant difference between the sexes in the time at which emergence began or ended, or in the mean hour of emergence.

IV. Acknowledgments

The authors are indebted to Dr. A. J. Nicholson and colleagues for criticism of the typescript.

V. References

Bateman, M. A. (1955).—The effect of light and temperature on the rhythm of pupal ecdysis in the Queensland fruit fly, *Dacus* (*Strumeta*) *tryoni* (Frogg.). *Aust. J. Zool.* **3**: 22–33.

Beard, R. L. (1940).—The biology of *Anasa tristis* de Geer with particular reference to the tachinid parasite *Trichopoda pennipes* Fabr. Bull. Conn. Agr. Exp. Sta. No. 440.

Brett, W. J. (1955).—Persistent diurnal rhythmicity in *Drosophila* emergence. *Ann. Ent. Soc. Amer.* **48**: 119–31.

Drake, C. J. (1920).—The southern green stink-bug in Florida. *Quart. Bull. Fla. State Pl. Bd.* **4**: 41–94.

Foley, J. C. (1945).—A study of average hourly values of temperature, relative humidity and saturation deficit in the Australian region from records of capital city bureaux. Bull. Bur. Met. Aust. No. 35.

O'Connor, B. A. (1950).—*Trichopoda pennipes* F. in Fiji and the British Solomon Islands. *Agric. J. Fiji* **21**: 63–71.

OBSERVATIONS ON THE BIOLOGY OF *HAEMAPHYSALIS BISPINOSA* NEUMANN (ACARINA : IXODIDAE) WITH PARTICULAR REFERENCE TO ITS MODE OF REPRODUCTION BY PARTHENOGENESIS

By K. C. Bremner*

[*Manuscript received November* 5, 1958]

Summary

Haemaphysalis bispinosa has been shown to reproduce by obligatory parthenogenesis. Male ticks were found to be scarce in both experimental and natural infestations, occurring in the ratio of one to approximately every 400 females. Dissections of five males showed that these produced no spermatozoa, irrespective of whether they were fed or not. Rates of development of the non-parasitic stages under controlled conditions are recorded.

I. Introduction

Haemaphysalis bispinosa Neumann is a three-host tick which has been recorded from a wide variety of mammalian hosts in Asia and Australasia (Nuttall *et al.* 1915). The distribution of this species in Australia is limited to the coastal areas of New South Wales and south-east Queensland, and heavy infestations have been observed on sheep and cattle (Seddon 1951; Roberts, personal communication).

The male of *H. bispinosa* was first described by Warburton (1908), and the records of Nuttall *et al.* (1915), Sharif (1928), Anastos (1950), and Kohls (1957) would indicate that males are common in India, south-east Asia, and Indonesia. Moreover, of 153 adults reared by Sapre (1945) from engorged females collected in India, 63 were males. On the other hand, Myers (1924), who studied the biology of this tick in New Zealand, observed that males were rarely found. Again Roberts (personal communication) and O'Sullivan (personal communication) have examined collections of this tick from many areas of south-east Queensland and have never seen males. This suggests that both bisexual and parthenogenetic races of *H. bispinosa* may occur, and Zhmaeva (1950) has demonstrated that the offspring of females collected in Siberia reproduced parthenogenetically when bred on rabbits and guinea pigs in the laboratory, with only females resulting from unfertilized ova.

The role played by males in the reproduction of the species in Queensland is unknown, and the following investigations were carried out in order to assess the sex ratio of the species, and the fertilizing capacity of the males. Details of the life cycle were also observed in the course of these studies. Rates of development under field conditions have been given by Myers (1924) and Legg (1926), and Sapre (1945) has studied the life history of this tick under controlled conditions.

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II. Methods

Engorged female *H. bispinosa* were collected from cattle at Mt. Tamborine, approximately 30 miles south of Brisbane, and were incubated at 30°C and 90 per cent. relative humidity in order to allow oviposition to proceed. Larvae hatching from eggs laid by these ticks were reared to the adult stage on rabbits or cattle. Rabbits were infested by placing ticks inside muslin bags glued over the ears with a resin–beeswax–lanolin mixture (16 : 3 : 1) (Gregson 1956). In experiments with cattle, calico bags were glued over the ears of tick-free animals which were maintained in isolation pens surrounded by moats filled with an acaricide. Zip-fasteners fitted to the calico bags permitted infestation and subsequent collection of engorged specimens.

Examinations of oviducts, seminal receptacles, and testes for the presence of sperm were made on squash preparations of these organs dissected out in 0·5 per cent. sodium chloride solution and stained with aceto-orcein (Bremner 1955).

Table 1

LIFE CYCLE OF H. BISPINOSA REARED ON RABBITS, AND INCUBATED AT 30°C AND 90 PER CENT. RELATIVE HUMIDITY AFTER ENGORGEMENT

Stage of Life Cycle	Time (days)	Stage of Life Cycle	Time (days)
Pre-oviposition period	5–6	Larvae moult	9–11
Oviposition	11–13	Nymphs engorge	4–7
Hatching of eggs	18–21	Nymphs moult	11–13
Larvae engorge	6–8	Adults engorge	9–15

III. Experimental

(*a*) *The Life History of* H. bispinosa

Table 1 shows the times of development of all stages of the life cycle of *H. bispinosa* reared on rabbits at room temperature, and maintained in an incubator at 30°C and 90 per cent. relative humidity after the engorgement of each feeding stage.

(*b*) *Frequency of Male Offspring*

Collections of engorged female ticks were made from dairy cattle at Mt. Tamborine on three occasions during the spring and summer of 1957–58. The eggs resulting from each collection of females were pooled, and 0·5-g samples were taken. The larvae hatching from these egg samples were reared on rabbits to the engorged nymphal stage, and the nymphs were separated into batches of approxi-

mately 50. These batches were incubated in separate test tubes, and the adults emerging after moulting were counted and sexed. The results are shown in Table 2. To ascertain whether larvae and nymphs developing under field conditions would give rise to similar sex ratios, engorged nymphs were collected from the same cattle in August 1958. The numbers of each sex moulting from these nymphs are shown in Table 2. Close examination of a cow from which these nymphs were collected, and which was moderately infested with female ticks in various stages of engorgement, revealed the presence of only one male.

These results show that the high proportion of female to male offspring persists throughout the breeding season of *H. bispinosa*, and suggest that sexual reproduction cannot be common within this species.

TABLE 2

FREQUENCY OF MALE OFFSPRING RESULTING FROM H. BISPINOSA COLLECTED FROM GRAZING CATTLE

Date of Collection	Stage Collected	Unengorged Adults Resulting	
		Male	Female
18. ix. 57	Engorged females	1	750
6. xii. 57	Engorged females	2	602
11. iii. 58	Engorged females	2	565
22.viii.58	Engorged nymphs	2	807

(*c*) *Reproduction by Parthenogenesis*

Two hundred females reared from eggs of the engorged females collected from cattle on September 18, 1957 (see Table 2), and which were from batches in which no males had appeared, engorged normally on cattle and laid viable eggs. A number of the larvae of this parthenogenetic generation were reared to the engorged nymphal stage on rabbits. Of 446 adults which moulted from these nymphs, two were males.

These results confirm that females of *H. bispinosa* can reproduce by parthenogenesis, and also show that fertilization by males is not necessary for the production of male offspring.

(*d*) *Examinations for the Presence of Spermatozoa*

(i) Unengorged, semi-engorged, and engorged female *H. bispinosa* were collected from naturally infested cattle at Mt. Tamborine. Squash preparations

were made from the oviducts and seminal receptacles of 20 ticks at each of these stages of engorgement, but no spermatozoa were seen. A further 20 engorged females collected at the same time were incubated and laid eggs from which larvae hatched in normal numbers. Ten engorged female *Boophilus microplus* Canestrini were also collected from these cattle, and spermatozoa were readily observed in squash preparations from each tick.

(ii) Squash preparations made from the testes of two males reared in the laboratory and incubated for 2 and 3 weeks respectively after moulting from the nymphal stage did not show any spermatozoa, whereas spermatozoa were readily demonstrated from reared males of the cattle tick, *B. microplus*, examined at comparable stages of development.

(iii) Three males and six unengorged females reared from nymphs engorging on a rabbit were placed on the ears of this host. By the following morning all ticks had attached, and 8–9 days later four of the females were engorged and had detached. The males changed their sites of attachment once, or at the most twice, but never attached in particularly close proximity to a female. Two males and the four engorged females were dissected on the ninth day following infestation. No spermatozoa were observed in squash preparations of testes, oviducts, or seminal receptacles.

The third male was allowed to remain attached to the host in company with the remaining two females, and died 11 days after infestation. No spermatozoa were found in the oviducts or seminal receptacles of the two female ticks.

(iv) Two males and six unengorged females which had emerged from engorged nymphs collected from naturally infested cattle were placed on the ear of a calf. The six females engorged and detached 9 days after infestation. No spermatozoa were detected in their oviducts or seminal receptacles. Neither of the males attached close to the females. One male was removed from the host 12 days after infestation, and no spermatozoa were found in its testes. The other male remained attached for 14 days when it died.

IV. Discussion

The results of these experiments indicate that "complete" parthenogenesis (White 1954) is the normal method of reproduction within the Mt. Tamborine strain of *H. bispinosa*. Spermatozoa were not present in the reproductive organs of females examined at various stages of engorgement on naturally infested cattle, or after engorgement on a rabbit and a calf to which male ticks were also attached. The absence of spermatozoa from the testes of all the males examined suggests that at no time are males capable of fertilizing ova. It seems unlikely that fertilization of female ticks could commonly occur off the host, as so few males were present in each generation, and all males examined proved infertile, irrespective of whether they were fed or unfed. Also, their indifference to unengorged or engorged females when placed in proximity to them either on or off a host suggests that males do not provide a behavioural releasing mechanism stimulating oviposition. The observation that females reared free of contact with males lay viable eggs in apparently normal numbers supports this suggestion.

The infrequent occurrence of males among the offspring reared from engorged females at intervals during the breeding season, and the fact that only rarely were males seen on grazing cattle at any time, indicate that cyclical parthenogenesis, with bisexual generations alternating with parthenogenetic ones, does not occur in this species in Queensland.

The abundance of male *H. bispinosa* in India and south-east Asia strongly suggests that in these areas this species reproduces sexually, and if this is so, both bisexual and parthenogenetic races of this tick exist. A number of ixodid species are known to be capable of parthenogenetic reproduction, namely *Amblyomma agamum* Aragão (Aragão 1912), *A. dissimile* Koch (Bodkin 1918), and *Hyalomma anatolicum* Koch (Pervomaĭskiĭ 1949), and Nuttall (1915) has induced the hatching of unfertilized eggs of *Rhipicephalus bursa* Canestrini & Fanzago by experimental means, but races within a species which differ in their modes of reproduction have not been reported previously.

The rates of development of the parasitic stages of *H. bispinosa* engorging on rabbits are in agreement with those given by Myers (1924) and Sapre (1945) for ticks engorging on cattle, the parasitic periods of larvae, nymphs, and adult females falling within the ranges of 4–10, 4–12, and 8–15 days respectively. This indicates that the rabbit is not an abnormal host for this tick. As the non-parasitic stages in this study were maintained at 30°C, rates of development shown for these stages are much shorter than those given by Sapre, who found larvae and nymphs took 17–25 and 28–34 days respectively to moult at 22°C.

The factors governing the production of male offspring, the reasons for the failure of spermatogenesis, and the mechanism by which the chromosome number is maintained within constant limits from generation to generation require cytological studies for their elucidation. The infrequent occurrence of males could be due to inconstancy of chromosome numbers within the species (White 1954). Preliminary observations on the cytology of Queensland Ixodidae have been made by the author and suggest that *H. bispinosa* is triploid. Suomalainen (1950) has pointed out that constant triploid parthenogenetic forms can arise naturally only in animals exhibiting an ameiotic type of parthenogenesis, and it would therefore appear unlikely that chromosomal reduction divisions occur during oogenesis in the strain of *H. bispinosa* studied.

V. Acknowledgment

The author is indebted to Dr. F. H. S. Roberts, C.S.I.R.O., Yeerongpilly, for his advice and interest.

VI. References

Anastos, G. (1950).—*Ent. Amer.* (N.S.) **30**: 28.

Aragão, H. (1912).—*Mem. Inst. Osw. Cruz* **4**: 96.

Bodkin, G. E. (1918).—*Parasitology* **11**: 10.

Bremner, K. C. (1955).—*Aust. J. Zool.* **3**: 312.

Gregson, J. D. (1956).—Canada Dep. Agric. Publ. No. 930.

Kohls, G. M. (1957).—Bull. Inst. Med. Res. F.M.S. No. 28. pp. 65–94.

Legg, J. (1926).—*Aust. J. Exp. Biol. Med. Sci.* **3**: 203.

Myers, J. G. (1924).—Bull. N.Z. Dep. Agric. No. 116.

Nuttall, G. H. F. (1915).—*Parasitology* **7**: 457.

Nuttall, G. H. F., Warburton, C., Cooper, W. F., and Robinson, L. E. (1915).—"Ticks: A Monograph of the Ixodoidea." Pt. 3. pp. 349–550. (Cambridge Univ. Press.)

Pervomaĭskiĭ, G. S. (1949).—*Zool. Zhurn.* **28**: 6.

Sapre, S. N. (1945).—*Indian J. Vet. Sci.* **15**: 47.

Seddon, H. R. (1951).—Serv. Publ. Dep. Hlth. Aust. Vet. Hyg. No. 7.

Sharif, M. (1928).—*Rec. Indian Mus.* **30**: 217.

Suomalainen, E. (1950).—*Advanc. Genet.* **3**: 193.

Warburton, C. (1908).—*Proc. Camb. Phil. Soc.* **14**: 517.

White, M. J. D. (1954).—"Animal Cytology and Evolution." 2nd Ed. 454 pp. (Cambridge Univ. Press.)

Zhmaeva, Z. M. (1950).—*Ent. Rev.* **31**: 21.

THE FEMALE UROGENITAL SYSTEM OF *PSEUDOCHEIRUS CONVOLUTOR* (MARSUPIALIA)

By J. Pearson* and J. M. de Bavay†

[*Manuscript received October* 16, 1958]

Summary

The female urogenital system of *Pseudocheirus convolutor* (Oken) has several interesting features which indicate that the genus should be placed among the less specialized members of the Phalangeroidea. Two of the three specimens examined had undergone parturition shortly before capture and an account is given of the histological processes involved in pseudovaginal parturition.

I. Introduction

The ring-tailed phalanger (genus *Pseudocheirus* Ogilby) has a wide distribution throughout Australia and New Guinea. Only one species, *Pseudocheirus convolutor* (Oken), is found in Tasmania where it is one of the commonest members of the marsupial fauna. In spite of frequent open seasons, when it is trapped for the skin market, this species appears to be holding its own and at present is in no danger of extinction. It is surprising, therefore, that little or nothing is known of the internal anatomy of this common species. The female urogenital system of *P. convolutor* which is dealt with in the present paper has not been described hitherto.

The following account of the female urogenital system of this species is based entirely upon an examination of serial sections of material taken from the following three specimens (and more particularly from the two parous females), namely: specimen No. 298, immature female; specimens Nos. 312, 324, parous females both with two very small "pouch embryos".

II. Description

The marsupial female urogenital system may be divided into four parts:

(1) The paired ovaries derived from mesodermal epithelium lining the coelom.

(2) Derivatives of the right and left Müllerian ducts of the embryo (mesodermal), viz. the paired fallopian tubes, the paired uteri, and the paired vaginae.

(3) The urinary system consisting of paired kidneys (metanephroi) and ureters, both mesodermal in origin, and a median bladder and urethra, both derived from the embryonic allantoic stalk and which are mainly endodermal in origin (Buchanan and Fraser 1918).

(4) The median urogenital sinus, which links the vaginae and urethra with the exterior, is endodermal in origin. The urogenital sinus, together with the hindgut, opens caudally into the ectodermal cloaca (not to be confused with the endodermal cloaca of the early embryo) (Buchanan and Fraser 1918).

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The paired ovaries, fallopian tubes, and uteri follow a consistent plan throughout the Marsupialia, a plan which differs in no important respect from the probable prototypal condition of the earliest viviparous mammals from which, presumably, both Metatheria and Eutheria have arisen. Thus in all marsupials there is a complete separation of the right and left uteri, in marked contrast to the variable eutherian pattern which ranges from two entirely separate uteri, comparable to the marsupial condition, to the single-chambered uterus of the Primates.

This uniformity makes it unlikely that a detailed comparative study of marsupial uteri would throw much light upon marsupial phylogeny, which has been one of the main purposes of a series of studies (Pearson 1945 and later). On the other hand, the more distal elements of the marsupial urogenital system, and particularly the vaginal complex, reveal considerable variability and departures from the basic plan of the more primitive forms. This progressive specialization of the marsupial urogenital system offers perhaps one of the most fruitful fields in this phylogenetic study.

III. Measurements

Plate 1, Figure 1, shows a horizontal section through the entire urogenital system of a fully grown parous female *P. convolutor* (specimen No. 324). The levels at which certain measurements were computed from the serial sections are indicated by the letters *A–F*. The measurements taken by this means are given in Table 1. Those of the uteri are not given, as the size of the uteri probably varies at different stages of the oestrous cycle, but the diagram gives the relative size of the uteri in the specimen at the time of its death.

(*a*) *Fallopian Tubes and Uteri*

The fallopian tubes are short and much convoluted. From the ostium abdominale, which is situated near the ovary, each tube passes forward for a distance of about 5 mm then makes an abrupt mediad and caudad turn to open into the anterior tip of the uterus.

The uteri are well-defined fusiform bodies about 25 mm long and 10 mm broad (specimen No. 312 shortly after parturition), each being splayed at an angle of about 25° to the antero-posterior axis of the body. Their posterior extremities lie close together on each side of the median line ensheathed in a common mass of connective tissue. The body of each uterus has spongy walls which are penetrated by complicated glandular extensions of the central uterine cavity (Plate 1, Fig. 1). This cavity is prolonged caudally into a narrow uterine neck. The right and left uterine necks run caudally, side by side, for a distance of about 4 mm and each opens into the vaginal cul-de-sac of its own side by means of the os uteri. Each os uteri is situated on the ventro-lateral aspect of a greatly distended uterine papilla which is attached to and forms part of the anterior portion of the median septum separating the right and left vaginae. The wall of each uterine papilla projects into the cavity of the anterior vaginal canal so as to reduce considerably the size of the canal. The tissue of each papilla is rendered spongy by the complicated invaginations of the

epithelium which lines the inner wall of each anterior vaginal canal. It is suggested that this spongy texture of the uterine papilla probably allows the os uteri to expand during parturition and thus facilitates the passage of the embryo from the uterus into the vagina.

In all three specimens the cavities of the uteri and uterine necks are lined by a single layer of cubical or columnar epithelium, which in the two parous specimens are transformed into a complicated system of uterine glands in the body of each uterus.

(*b*) *Vaginal Complex*

This consists essentially of the right and left vaginae, which are derivatives of the two Müllerian ducts of the embryo. The most distal parts of the two vaginae lie posterior to the two uterine necks. From this level each vagina pursues an

TABLE I

MEASUREMENTS OF THE FEMALE UROGENITAL SYSTEM OF P. CONVOLUTOR

Antero-posterior Length of:	Specimen No. 298 (pouch young)		Specimen No. 312 (parous)		Specimen No. 324 (parous)	
	Length (mm)	Percentage of *A–E*	Length (mm)	Percentage of *A–E*	Length (mm)	Percentage of *A–E*
Vaginal complex (*A–E*)*	3·72	100	16·12	100	20·85	100
Anterior vaginal canal (*A–B*)	0·63	16·9	2·35	14·5	3·98	19·1
Cul-de-sac (*B–D*)	0·45	12·1	5·12	31·8	5·30	25·4
Urethra (*C–E*)	2·94	79·0	9·05	56·1	12·37	59·3
Pseudovagina (*D–E*)	2·64	71·0	8·65	53·7	11·57	55·5
Urogenital sinus (to tip of clitoris) (*E–F*)	?	?	*c.*27·0	167·0	*c.*32·0	153·5

*See Plate 1, Figure 1, for the positions of *A*, *B*, *C*, *D*, *E*, and *F*.

S-shaped course to the urogenital sinus, in accordance with the usual marsupial pattern. Each vagina consists of (i) a proximal region, the median vaginal cul-de-sac; (ii) an intermediate section, the anterior vaginal canal, forming the second loop; and (iii) a distal section, the lateral vagina which passes caudally and enters the unpaired urogenital sinus.

(i) *Median Vaginal Culs-de-sac.*—In specimen No. 298, which is immature, the two culs-de-sac are entirely separate from each other, a condition which is found in all immature females throughout the Marsupialia. In specimens Nos. 312 and 324, however, which are both parous females, the right and left culs-de-sac lie in close contact medially, separated by a septum which is stout in its anterior region but which gradually becomes more tenuous in the posterior half. In this posterior region the septum has been perforated and provides the only connexion between the vaginal

cavities of the right and left sides (Plate 2, Fig. 8). A comparative examination of the female urogenital system of marsupials justifies the assumption that this breach is usually made at the first parturition and, once made, persists throughout life. (In some of the more primitive forms, however, the right and left vaginae are believed to remain separate throughout life.)

The culs-de-sac are lined by a single layer of cubical or columnar epithelium interspersed by scattered pockets of stratified epithelium. They are relatively short and have an antero-posterior length of about 5 mm (Nos. 324 and 312).

The brevity of the culs-de-sac results in an inordinately long "pseudovagina" and these features lend support to the claim that *Pseudocheirus* is one of the least specialized genera of the Phalangeroidea.

(ii) *Anterior Vaginal Canals.*—Anterior to the level of the os uteri each cul-de-sac passes imperceptibly into an anterior vaginal canal which runs forward at a right angle to the antero-posterior axis of the body (Plate 1, Figs. 1 and 2). This is the second component of the vaginal complex. In specimen No. 324 its lumen is greatly reduced by the distension of the uterine papilla and has a crescentic appearance in transverse sections (Plate 2, Figs. 5, 6). On the other hand, in specimen No. 312, in which some of the vaginal cavities are greatly distended, the lumen of the anterior vaginal canal is not reduced as much as in No. 324. The epithelium of the anterior vaginal canals consists of a single layer of cubical or columnar epithelium in which the cells readily take haematoxylin stain. At its most distal extremity each anterior vaginal canal bends outward and passes into the third component of the vagina, the lateral vagina. It is impossible to say where one ends and the other begins, but it is convenient to assume arbitrarily that the anterior vaginal canal terminates at the apex of the second bend. An examination of the epithelium does not clarify the question as there is an intermediate zone in which there is a transition from the simple epithelium of the anterior vaginal canal to the stratified epithelium so characteristic of the lateral vagina.

In No. 324 (Plate 3, Fig. 9) the epithelium covering the floor, and to a less extent the lateral walls, of the anterior vaginal canal has the same complicated spongy arrangement as do the uterine papillae in transverse section. In No. 312, however, in which the vaginal cavities are distended to a marked degree, these epithelial invaginations of the floor and lateral walls of the canal are hardly perceptible (as might be expected). In other words, the walls of the canal are deeply corrugated in the relaxed condition and smooth in the distended condition.

(iii) *Lateral Vaginae.*—Commencing at the apex of the second bend, each lateral vagina constitutes the most distal portion of the vaginal complex. It runs caudally and opens into the anterior extremity of the urogenital sinus which also receives the urethra. The antero-posterior extent of each lateral vagina is represented by *A–E* in Plate 1, Figures 1 and 2.

An important feature is the persistence of a vestigial Wolffian duct near the caudal extremity of each lateral vagina. This interesting vestige has already been reported by us in other marsupial genera (Pearson and de Bavay 1951, 1953). This

may be an indication of the unspecialized condition of the female urogenital system of this genus.

A detailed account of the intimate structure of the posterior region of the lateral vaginae will be given in a later paper in which the occurrence of a vestigial Wolffian duct in parous females of certain marsupials will be fully considered. Plate 1, Figures 1 and 2, shows the presence of a Wolffian remnant in the lateral vagina.

As is universally the case in adult female marsupials the lateral vaginae are lined by stratified epithelium.

(*b*) *Urethra*

The ureters enter the neck of the bladder at a level slightly anterior to the caudal termination of the cul-de-sac (Plate 3, Fig. 10). The urethra opens at this level and conveys the contents of the urinary bladder to the urogenital sinus and thence to the exterior. The urethra is a relatively long median tube which runs backward ventral to the position of the pseudovagina (Plate 3, Figs. 11 and 12). It has a length slightly more than one half of the total antero-posterior length of the vaginal complex in adult females and has a width of about 4 mm at its anterior extremity and gradually tapers to about 2 mm at its point of entry into the urogenital sinus. It is lined throughout with stratified epithelium. Both bladder and urethra may be regarded as remnants of the embryonic allantoic stalk. In the course of development the two ureters which ultimately open into the neck of the bladder have shifted from their original position as outgrowths from the Wolffian ducts. By a process of allometric growth they first take up a position independent of the Wolffian ducts in the anterior wall of the urogenital sinus at the base of the allantoic stalk. Later, by a further stage of unequal growth, the allantoic stalk lengthens between the urogenital sinus and the roots of the ureters to form the urethra, which in the adult links the urinary bladder with the urogenital sinus (see Buchanan and Fraser 1918).

(*c*) *Urogenital Sinus*

This is a median tube which receives the two lateral vaginae and the median urethra at its anterior extremity and opens into the cloaca. Its total length (*E–F*) is more than $1\frac{1}{2}$ times the antero-posterior length of the vaginal complex (*A–E*) (Plate 1, Fig. 1).

IV. Notes on Parturition

It was evident that Nos. 312 and 324 had been captured only a short time after parturition had taken place as two very small embryos were present in the pouch of each specimen. An examination of serial sections later confirmed this.

No. 324 was captured alive and No. 312 was "dead when taken but fresh". The urogenital organs of each parent were removed on the spot and fixed in Bouin's fluid. The histological details of both series were clear and completely adequate and revealed a very interesting and significant state of affairs.

In No. 312 the right uterus was larger than the left and, moreover, contained irregular masses of cells, presumably foetal remnants, which were entirely lacking

in the left uterus.* This might suggest that both foetuses were derived from the right uterus only. Doubts about this, however, are raised by the presence of a few scattered cells lying freely in the cavity of the left uterine neck, and also by the occurrence of irregular groups of cells scattered throughout the cul-de-sac and anterior vaginal canal of *both* right and left sides. The presence of these disintegrated tissues in both right and left vaginae may be due merely to the free exchange of vaginal fluid and contents across the ruptured intervaginal septum.

(*a*) *Break in the Vaginal Epithelium at Parturition*

In his observations on *Perameles*, Hill (1899) did not notice any signs of a break in the continuity of the vaginal epithelium, and he concluded that when the break did occur, as it undoubtedly must, the subsequent repair of the epithelial tissue took place very rapidly. It is all the more fortunate that in the two specimens of *Pseudocheirus* (Nos. 312 and 324) the terminal wall of the culs-de-sac was badly torn and repair of the vaginal epithelium had not yet been effected. So far as can be ascertained these are the only two cases in which this important phase has been observed.

Plate 4, Figure 14, shows the broken condition of the epithelium in the terminal portion of the conjoined culs-de-sac. Three regions of columnar epithelium may be clearly identified (*a*, *b*, and *c*). Between *a* and *b* and between *b* and *c*, the epithelium appears to have disintegrated to a considerable degree, but attention is drawn particularly to the wide gap on the right-hand side of the photograph between *a* and *c*, where the epithelium has completely disappeared. There can be no question that it was at this point that the foetuses passed out of the vaginal culs-de-sac into the surrounding connective tissue. A somewhat similar condition is found in specimen No. 312 where there is one extensive break in the continuity in the epithelium and a number of isolated patches of epithelium. In this case the cavity of the culs-de-sac contains some extravasated blood which may be due to the violent rupture of the epithelial wall, or to the separation of the foetal membranes which are shed by the foetuses in the course of their hazardous journey to the exterior. This degeneration of the vaginal wall is probably due, in part at least, to hormonal action, and conceivably the internal pressure of the vaginal fluid is brought to bear upon the weakened tissues when the foetuses approach this point in their journey.

(*b*) *Pseudovagina*

When the foetuses emerge from the vagina they enter a compact zone of connective tissue immediately dorsal to the urethra and extending from the culs-de-

*Among marsupials there appears to be some difference in the fate of the foetal membranes:

Didelphys virginiana.—From Hartman's account (1952, p. 84) it appears that the membranes and amniotic fluid emerge with the embryo and are consumed by the mother when freeing the embryo of them.

Dasyurus quoll.— . . . "the foetal membranes, in greater part, remain *in situ* and are gradually absorbed through the agency of the maternal leucocytes" (Hill 1900).

Perameles.—From Hill's account (1899) it appears that the allantoic stalks are retained in the uteri and median vaginae, where they are absorbed, and in the pseudovagina where they become fibrosed and incorporated in the connective tissue.

sac to the anterior extremity of the urogenital sinus. The foetuses are able to force their way through this connective tissue by reason of the impetus already given them by the pressure of the vaginal fluid. Here again hormonal action may assist parturition by "softening" the connective tissue through which the foetuses have to pass. The result is that a direct median path is created which remains open for some time after its purpose has been served. This temporary median split in the connective tissue through which the foetuses pass is the pseudovagina, first named by Hill (1899). Hill and Fraser (1925) stated "we are inclined to think that this median mode of birth will ultimately be found to hold good for the whole of the Marsupialia and in that event it would come to constitute a class character".*

In specimen No. 324 the pseudovagina has a length of 11·5 mm. The cavity of the anterior half is irregular and ill defined, and presumably is in the process of reverting to the normal homogeneous state in which there is no median passage. This contrasts sharply with the condition of the posterior half in which there is a well-defined canal, tri-radiate in section, having clear-cut walls which, however, are not lined with epithelium. The posterior extremity of the vagina is in close contact with one of the sinus horns of the urogenital sinus, where there is clear evidence of a rupture of the epithelial wall which is in process of repair (Plate 4, Figs. 15 and 16). There would appear to be no doubt that this is the point at which the foetuses made their way from the posterior tip of the pseudovagina into the urogenital sinus.

V. Discussion

The most important features of the vaginal complex of *P. convolutor* are as follows:

(i) The general plan of the vaginae follows that of the more primitive marsupials, i.e. the persistence of the double bend.

(ii) The antero-posterior length of the vagina is considerably less than that of the urogenital sinus.

(iii) The vaginal culs-de-sac are relatively short, and, in consequence, there is a very long pseudovagina.

(iv) Right and left culs-de-sac remain *almost* completely separated after parturition.

(v) A clearly defined vestigial Wolffian duct persists throughout life.

(vi) To a great degree the culs-de-sac and the anterior vaginal canals retain a single-layered epithelium.

(vii) Twin births are almost universal (it has been stated that as many as six young may be born but not more than two survive).

The above characteristics are possessed by the least specialized marsupials and justify the conclusion that this genus is one of the more primitive members of the Phalangeroidea.

*There are, however, a few cases in the Phalangeroidea in which, after the first parturition, a permanent connexion persists between the culs-de-sac and the urogenital sinus. Also, it has been shown (Pearson 1945) that in the only recorded cases of parturition in the rat-kangaroo, *Potorous*, the lateral vagina was used as a birth passage.

Most systematists, using dental and pedal characters only, have considered *Pseudocheirus* to be closely related to the brush-tailed phalanger *Trichosurus*, and for this reason Thomas (1888), Iredale and Troughton (1934), and Troughton (1951) placed both genera in the subfamily Phalangerinae. On the contrary, Tate (1945), after an exhaustive study of cranial and dental characters, concluded that *Pseudocheirus* was more closely allied to the koala, *Phascolarctos*, and placed the two genera in the subfamily Phascolarctinae.

Tate's view would accord more closely with the conclusions which have been reached in the course of the present series of investigations.

The female system of *Phascolarctos* is relatively primitive in two important respects, viz. the complete separation of right and left culs-de-sac throughout life, and the presence of short culs-de-sac with a correspondingly long pseudovagina. On the other hand, in *Trichosurus* the two vaginal culs-de-sac lose their dividing septum at the first parturition to form a capacious single-chambered cul-de-sac which extends as far as the urogenital sinus so that the pseudovagina is almost non-existent.

VI. Acknowledgments

It is wished to gratefully acknowledge the painstaking assistance of Mr. F. Roberts, Teachers' College, Armidale, N.S.W., in the preparation of Plates 1 and 2. Thanks are also due to Professor A. F. O'Farrell, Associate Professor A. Stock, and to Miss Rosemary Cullen, all of the Zoology Department, University of New England, for helpful comments and suggestions.

VII. References

Buchanan, G., and Fraser, E. C. A. (1918).—The development of the urinogenital system in the Marsupialia with special reference to *Trichosurus vulpecula*. *J. Anat.* **53**: 35–95.

Hartman, C. G. (1952).—"Possums." (University of Texas Press: Austin.)

Hill, J. P., (1899).—Contributions to the morphology and development of the female urinogenital organs in the Marsupialia. *Proc. Linn. Soc. N.S.W.* **24**: 42–82.

Hill, J. P. (1900).—On the foetal membranes, placentation, and parturition of the native cat (*Dasyurus viverrinus*). *Anat. Anz.* **18**: 364–73.

Hill, J. P., and Fraser, E. C. A. (1925).—Some observations on the female urogenital organs of the Didelphidae. *Proc. Zool. Soc., Lond.* **1925**(1): 189–219.

Iredale, T., and Troughton, E. Le G. (1934).—A check-list of the mammals recorded from Australia. Mem. Aust. Mus. No. 6.

Pearson, J. (1945).—The female urinogenital system of the Marsupialia with special reference to the vaginal complex. *Pap. Roy. Soc. Tasm.* **1944**: 71–98.

Pearson, J., and de Bavay, J. M. (1951). The female urogenital system of *Antechinus* (Marsupialia). *Pap. Roy. Soc. Tasm.* **1950**: 137–42.

Pearson, J., and de Bavay, J. M. (1953).—The urogenital system of the Dasyurinae and the Thylacininae (Marsupialia, Dasyuridae). *Pap. Roy. Soc. Tasm.* **87**: 175–99.

Tate, G. H. H. (1945). Results of the Archbold expeditions. No. 54. The marsupial genus *Pseudocheirus* and its subgenera. Amer. Mus. Novit. No. 1287.

Thomas, O. (1888).—"Catalogue of Marsupials and Monotremes." (British Museum: London.)

Troughton, E. Le G. (1951).—"Furred Animals of Australia." (Angus & Robertson: Sydney.)

FEMALE UROGENITAL SYSTEM OF PSEUDOCHEIRUS CONVOLUTOR

A Anterior limit of vagina

B Os uterus

C Entry of ureters into urinary bladder

D Posterior limit of vaginal culs - de - sac

E Anterior limit of u.g.s.

F Posterior limit of u.g.s. (level of tip of clitoris)

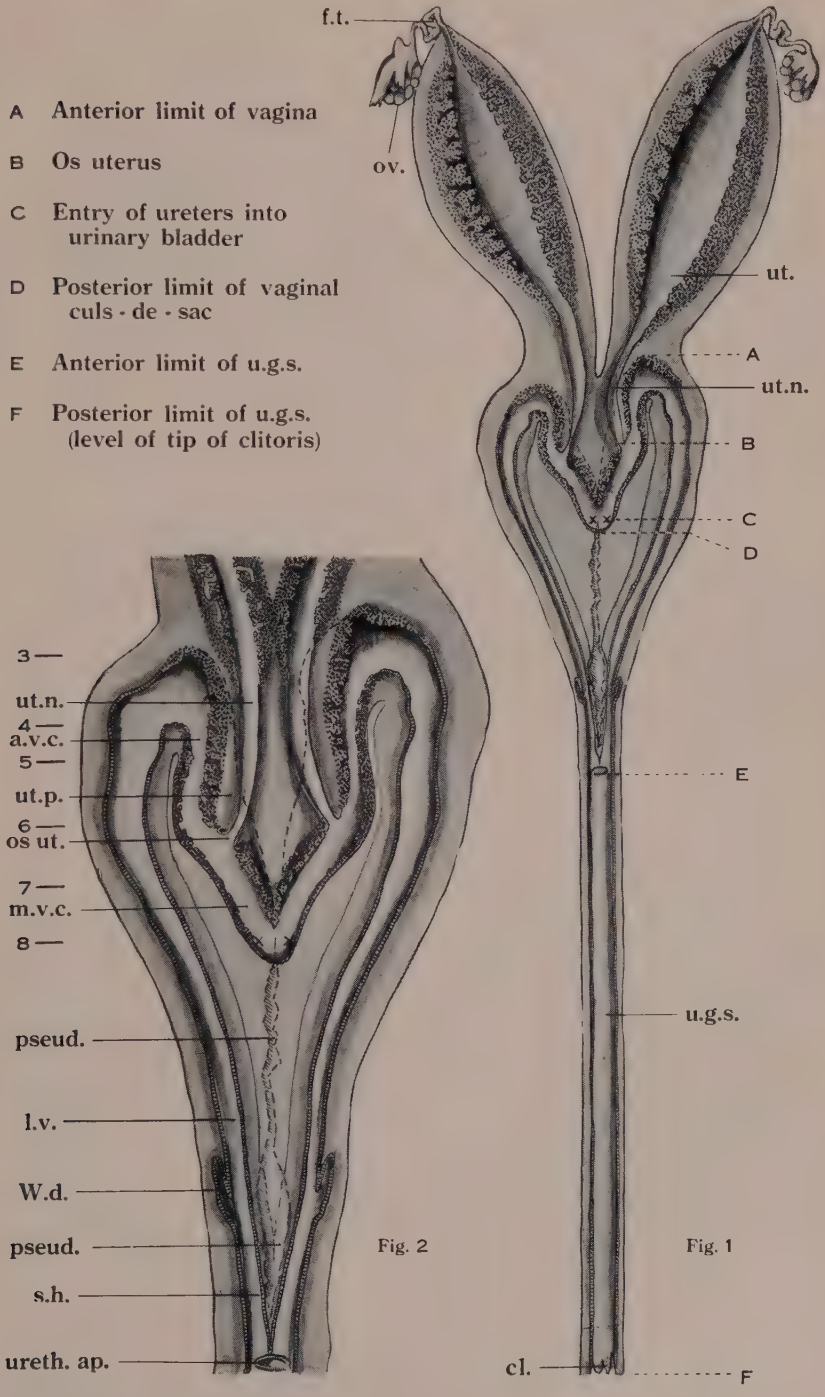

Fig. 2

Fig. 1

FEMALE UROGENITAL SYSTEM OF PSEUDOCHEIRUS CONVOLUTOR

Fig. 3

Fig. 4

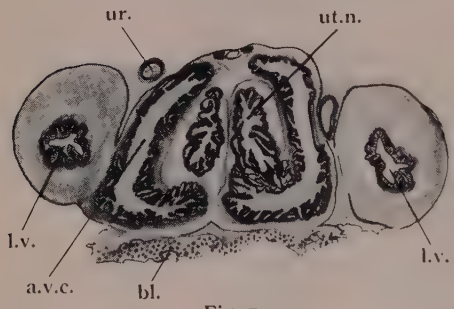

Fig. 5

Fig. 6

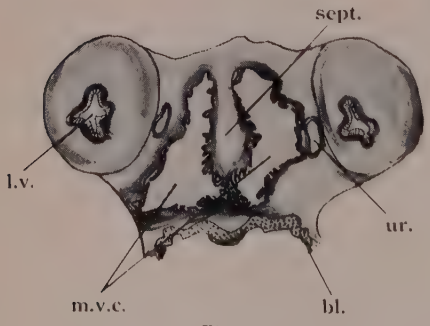

Fig. 7

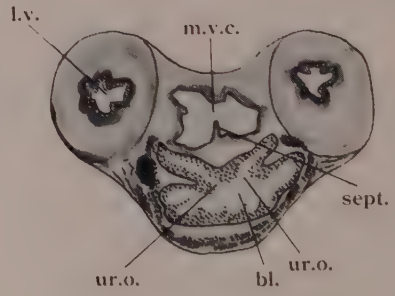

Fig. 8

FEMALE UROGENITAL SYSTEM OF PSEUDOCHEIRUS CONVOLUTOR

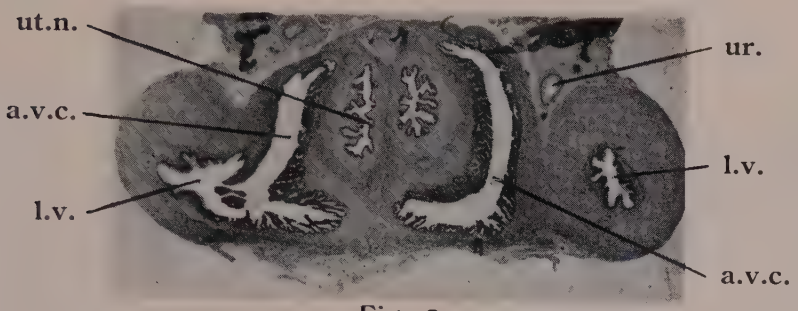

Fig. 9

Fig. 10

Fig. 11

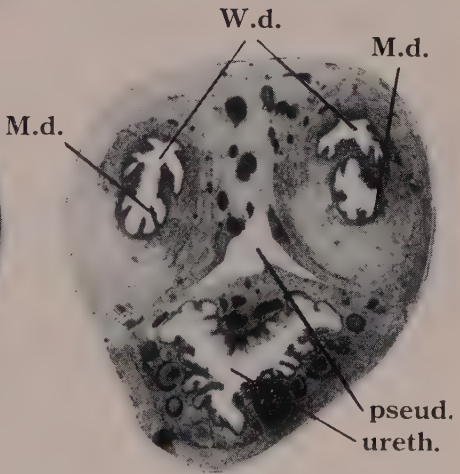

Fig. 12

FEMALE UROGENITAL SYSTEM OF PSEUDOCHEIRUS CONVOLUTOR

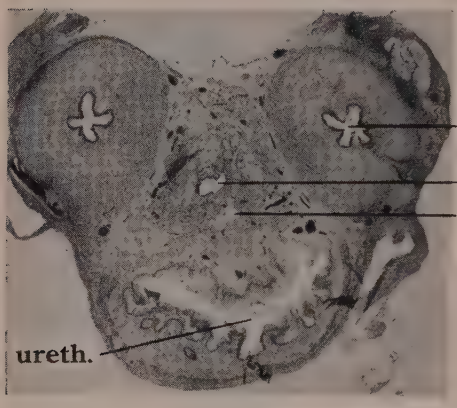

Fig. 13

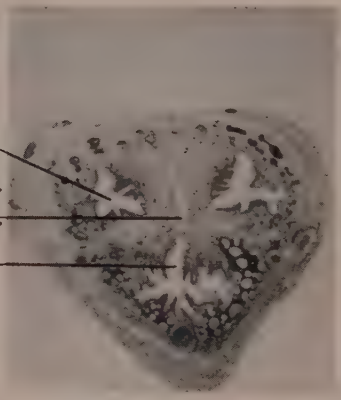

Fig. 15

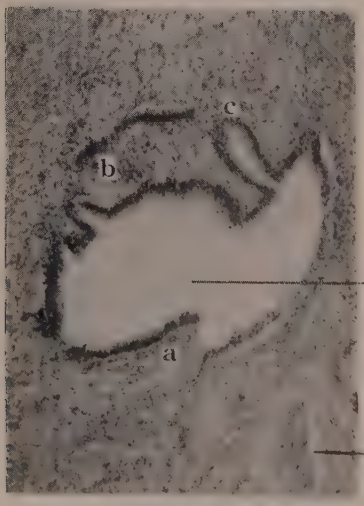

Fig. 14

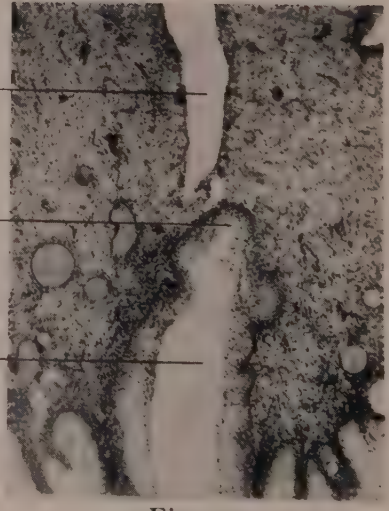

Fig. 16

Explanation of Plates 1–4

a.v.c., Anterior vaginal canal; *bl.*, bladder; *cl.*, clitoris; *l.v.*, lateral vagina; *M.d.*, Müllerian duct; *m.v.c.*, median vaginal canal; *os ut.*, os uterus; *pseud.*, pseudovagina; *rupt. sept.*, ruptured septum; *sept.*, septum between right and left culs-de-sac; *s.h.*, sinus horn; *u.g.s.*, urogenital sinus; *ur.*, ureter; *ur. o.*, opening of ureter into bladder; *ureth. ap.*, urethral aperture into urogenital sinus; *ut.*, uterus; *ut. n.*, uterine neck; *ut.p.*, uterine papilla; *W. d.*, Wolffian duct; *x*, point indicating probable course of parturition between pseudovagina and sinus horn

Plate 1

Fig. 1.—Horizontal section through the urogenital system of specimen No. 324. The letters *A–F* indicate the various levels at which antero-posterior measurements were taken (see Table 1). × 2·7.

Fig. 2.—Horizontal section through the vaginal complex of specimen No. 324. The numbers 3–8 indicate the levels at which the sections illustrated in Plate 2, Figures 3–8, respectively were cut. × 4·5.

Plate 2

Diagrammatic transverse sections through the vaginal region of specimen No. 324. × *c.*5.

Fig. 3.—Showing on the left side the extreme anterior tip of the anterior vaginal canal, on the right side the connexion between the anterior vaginal canal and the lateral vagina.

Fig. 4.—Showing on the left side a similar condition to the right side of Plate 2, Figure 3; on the right side the anterior vaginal canal and the lateral vagina are shown as separate ducts (see also Plate 3, Fig. 9).

Fig. 5.—Both sides show a condition similar to the right side of Plate 3, Figure 4.

Fig. 6.—This section is cut at the level of one *os uteri* and slightly anterior to that of the other (see also Plate 3, Fig. 10).

Fig. 7.—The two median vaginal culs-de-sac are separated by a stout septum.

Fig. 8.—Showing the entry of the two ureters into the neck of the bladder. The two culs-de-sac communicate with each other through a break in the septum.

Plate 3

Photomicrographs of transverse sections through the vaginal region of specimen No. 324

Fig. 9.—Transverse section cut at level 4 of Plate 1, Figure 2 (see also Plate 2, Fig. 4). × *c.* 6.

Fig. 10.—Transverse section cut at level 6 of Plate 1, Figure 2 (see also Plate 2, Fig. 6). × *c.* 6.

Fig. 11.—Transverse section cut at level of the vestigial Wolffian ducts free from the respective Müllerian ducts. The pseudovagina is seen. × *c.* 12.

Fig. 12.—Transverse section cut slightly posterior to Plate 3, Figure 11, showing the fusion of the left Wolffian and Müllerian ducts. The pseudovagina is larger than in Plate 3, Figure 11. × *c.* 12.

Plate 4

Photomicrographs of transverse sections through the vaginal region of specimen No. 324

Fig. 13.—Transverse section at the level of the extreme caudal tip of the fused right and left culs-de-sac. × *c.* 7.

Fig. 14—Enlarged portion of Plate 4, Figure 13, showing the condition of the epithelium at the point of egress of the foetuses from the cul-de-sac. × *c.* 53.

Fig. 15.—Transverse section at the level where the foetuses passed from the pseudovagina into the urogenital sinus. × *c.* 8.

Fig. 16.—Enlarged portion of Plate 4, Figure 15, showing a point of contact between the pseudovagina and one of the sinus horns of the urogenital sinus which marks the probable course of parturition. × *c.* 65·5.

THE STRUCTURE AND FUNCTION OF THE EPIDIDYMIS

II. THE HISTOGENESIS OF THE RAT EPIDIDYMIS

By B. L. Reid*

[*Manuscript received August* 7, 1958]

Summary

Observations were made on the epididymides of young white rats of the following ages: 3, 21, 28, 32, 37, 39, 56, 72, 96, 110 days.

Both efferent ducts and epididymal duct are undifferentiated at 21 days with a similar cuboidal epithelium. The connective tissue coat of the efferent ducts is one cell thick whereas that of the epididymal duct is two or three cells thick. The possible involvement of the connective tissue in the process of histogenesis is discussed.

Differentiation within the epididymal duct commences at 28 days when the epithelium in the cephalic portion is tall and that in the caudal portion of the head and remainder of the tail is tall with isolated segments of low columnar epithelium. The latter epithelium is associated with a wider lumen which evidently becomes continuous down the duct. In the efferent ducts at this stage ciliated cells have appeared.

Differentiation of the cephalic portion of the head is completed rapidly by the 37th day but that of the caudal portion of the head and tail of the organ is completed only at the 96th day. In certain zones, histodifferentiation is accompanied by obvious nuclear differentiation.

Spermatozoa first appear in the testis at 56 days but do not enter and fill the epididymal ducts until 72 days. There is evidence of an outflow of fluid from the testis which carries spermatocytes and spermatids into the duct at 32 days.

The changes in the epithelium of the efferent duct, the epididymis, and the deferent duct from the 3rd to the 110th day are tabulated.

I. Introduction

The histogenesis of the epididymis appears to have received little attention from histologists in the past. This may well have been due to the absence of an accurate knowledge of the succession of histological types making up the lining epithelium of the ducts. Histological examination of the adult organ (Reid and Cleland 1957) revealed a great complexity in these ducts and it was felt that a study of ontogeny might shed further light on the origin of the diverse epithelial types. Further, with their relative delay in the histogenesis of later stages, the genitalia of mammals represent favourable material, readily available from the young animal, for studies on cytodifferentiation in general.

Benoit (1926) appears to have been amongst the earlier workers in this field to examine the foetal and young mouse epididymis. He noted the precocious development of the efferent ducts, which assume an adult-like appearance within

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5 days after birth. Cilia were present at the 5th day and secretion granules appeared about the 15th day. He noted this early differentiation of ciliated cells in a variety of mammals, tracing their origin back to prenatal stages. He found that an increase in the number of mitochondria preceded other more obvious cytological differentiation such as stereocilia or brush border. He also drew attention to the decreasing nucleocytoplasmic ratio amongst epithelial cells of the efferent ducts and head of the organ during development from embryonic to adult stages.

Pfeiffer (1928) in a lengthy study of the development of the human epididymis noted the precocity of the efferent ducts, and of ciliated cells within them, and of the fluid outflow from the testis, ascribing a secretory and possible resorptive function to the efferent ducts even at birth. He also noted the delayed differentiation in the tail.

Tao and Eaton (1954), in a study of young goats, refer to a long period of 70 days after birth in which the epithelium of the head and tail of the organ are similar. This is followed by the development of pseudostratified epithelium with tall columnar epithelium in the head and cuboidal in the tail. They noted that stereociliated cells were present before mature sperms were found in the head of the organ. They figure the thick connective tissue coat in the 30-day-old animal.

The terminology used in this paper with reference to the various histological zones of the epididymis follows that proposed by Reid and Cleland (1957).

II. Materials and Methods

Testes and epididymides from white rats were fixed in Helly's fluid to which 5 per cent. acetic acid and 10 per cent. formalin were added. Two animals in each of the following ages (in days) were selected at random from a normal animal house colony: 3, 21, 28, 32, 37, 39, 56, 72, 96, 110. The organs were serial-sectioned at 8μ and stained with haematoxylin and eosin. When it was established that the most active stage of histogenesis was about 32 days, further specimens from this age group were fixed in Helly's fluid and postchromated at 37°C in 2 per cent. potassium dichromate for 3 days and in osmium fixative by the Ludford (1925) modification of the Mann-Kopsch technique.

The most convenient method of assessing the relative mitotic activity of the tissue under observation was by a simple count of mitotic figures per 100 tubule cross sections. Counts per length of tubule by reconstruction from serial sections were thought to be less valid in a tube where cell size was under constant change during the growth period.

III. Results

A summary of the observations described in this section may be found in Table 1.

(*a*) 3 *Days*

The general plan of the epididymis is apparent by the 3rd postnatal day and five efferent ducts can be discerned whose cephalic ends are convoluted into the anlagen of the coni vasculosi and whose terminal ducts join the epididymal canal. The conus region is very small.

TABLE 1

SUMMARY OF THE MAIN CHANGES DURING HISTOGENESIS OF THE RAT EPIDIDYMIS

Day	Efferent Duct	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Deferent Duct
3		Undifferentiated simple cuboidal to low columnar epithelium						Three to four layers of concentric lamina of connective tissue
	Thin connective tissue investment	Thicker connective tissue investment						
21	As for 3 days	Undifferentiated simple cuboidal to low columnar epithelium						Pseudostratified columnar epithelium not folded, six to eight layers of connective tissue
		Connective tissue investment loose, two to three layers thick		Connective tissue investment tightly packed, one to two layers thick		Connective tissue investment as for zones 1 and 2		
28	Differentiation by clarity of cytoplasm in initial zone and appearance of ciliated cells	Tall epithelium of stratified columnar type; mainly two rows of nuclei			Alternating segments of tall and short stratified columnar epithelium	Stratified columnar epithelium; two to four rows of nuclei; stellate lumen		Connective tissue laminae recognizable as smooth muscle

TABLE 1 (*Continued*)

Day	Efferent Duct	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Deferent Duct
32	Advanced differentiation of terminal and initial zones. Prominent coni vasculosi region	Tall columnar epithelium of definitive structure, about 60 per cent. of definitive height. Two rows of nuclei	Shorter columnar epithelium with scant apical vacuoles	Similar height columnar epithelium to zone 2 without apical vacuoles	As for 28 days	As for 28 days		As for 28 days. Folded epithelium as for adult
37	As for 32 days. Definitive pattern established	As for 32 days	As for 32 days. Increasing numbers of cells with apical vacuoles	Increased volume. Epithelium lower at 35 μ	As for 28 days	As for 28 days	Becomes differentiated from vas deferens at 39 days as pale cytoplasm with several rows of nuclei	As for 32 days
56	As for 32 days	Definitive pattern established	Definitive pattern established	As for 37 days	At junctional area with zone 3 is tall stratified columnar epithelium More caudally it is low columnar. Juxtanuclear vacuoles appear	As for 28 days	Inactive clear cells appear. Two to three rows of nuclei, occasionally one row	As for 32 days

TABLE 1 (*Continued*)

Day	Efferent Duct	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Deferent Duct
72	As for 32 days	Sperm present in all zones of adult distribution						
		As for 56 days	As for 56 days	As for 56 days	Small residue of tall epithelium cranially. Remainder is low columnar	Tall columnar epithelium. One to two rows of nuclei. Rounded lumen	As for 56 days. The majority of oval nuclei now have long axes parallel to surface	As for 32 days
96	As for 32 days	As for 56 days	As for 56 days	As for 56 days	Gross increase in volume. Nuclei becoming irregular in shape	Low columnar epithelium of definitive type. Irregularity of shape of nuclei	Flattening of cells and nuclei. Nuclei irregular in shape	As for 32 days
110	As for 32 days	As for 56 days	As for 56 days	As for 56 days	As for 96 days	As for 96 days	As for 96 days. Many nuclei show peripheral distribution of chromatin	As for 32 days

The canal is a miniature of the adult form and comprises a head distinguished by increased folding of the canal and by subdivision by connective tissue septa into lobules. The middle portion of the canal is constricted into a waist in which the canal is reduced to a fairly straight tube. In the tail the canal is little convoluted and soon gives way to the vas deferens which is recognized, even at this early stage, by a thick coat of 10–12 layers of concentric connective tissue cells.

Throughout their extent the ducts are lined with a single layer of cells which are all alike and relatively unspecialized, having the following characteristics (Plate 1, Figs. 1 and 2):

(i) The cell shape is ovoid, about 20 μ high.

(ii) The cytoplasm, like that of other embryonic cells, stains darkly and is featureless. The nucleus is oval and its contour is smooth. It occupies most of the volume of the cell. There are usually from one to three prominent nucleoli.

(iii) The connective tissue investment of this undifferentiated lining layer, however, varies. In the efferent ducts (Plate 1, Fig. 1) there are one or two concentric layers of plump spindle cells but in the canal itself there are three or four layers whilst in the vas deferens there are up to 10 such layers closely packed.

The lumen diameter of the tubes is approximately constant throughout at 15 μ.

Some 32 per cent. of tubule cross sections in the three zones exhibit a mitotic figure.

Testis.—The seminiferous tubules are about 50 μ in diameter and contain two cell types—the primitive spermatogonia and the gonocytes. The former are distributed peripherally forming the wall of the tubule and are about 20 μ tall. The latter are usually within the lumen of the tubule and are about twice the diameter of the spermatogonia.

(*b*) 21 *Days*

The overall degree of differentiation is little different from the 3-day epididymis both as regards shape of the organ and histological characteristics of the epithelial cells.

The connective tissue investment is thinner and there are qualitative differences in certain parts. In the efferent ducts the investment is one cell thick and tightly clothes the tube (Plate 1, Fig. 3). In the cephalic portion of the head, in the presumptive tall epithelial zones of the adult, the cells are more loosely packed and two or three layers thick (Plate 1, Fig. 4). The nuclei are large. In the more caudal portion of the head in the presumptive low epithelial zones of the adult, the cells are tightly packed and one or two layers thick.

The middle portion and tail of the organ have an investment similar to the cephalic portion of the head. The pre-vas-deferens portion has six to eight layers of concentric spindle cells, much as in the 3-day animal. In fact, this configuration of connective tissue cells is so typical as to distinguish the pre-vas-deferens zone quickly at all ages.

The mitotic rate per tubule has fallen off considerably to six mitotic figures per 100 tubule cross sections and is approximately equal in efferent ducts and canal portions. About 50 per cent. of the figures are in prophase, the remainder equally divided between metaphase and telophase.

Testis.—The testis is similar in its developmental stage to that of the 3-day-old animal except that the number of gonocytes is greatly reduced to 1 or 2 per cent. of tubule cross sections. The cell population of the tubules is more uniform in size and thus presents a markedly homogeneous appearance. Many nuclear shapes are found amongst the spermatogonia, e.g. comma, ovoid, round.

(*c*) 28 *Days*

By the 4th week some degree of cell differentiation has become apparent in the epididymis and testis.

In the efferent ducts ciliated cells have made their appearance. The cell is little different in shape from non-ciliated cells but possesses an ill-defined row of granules at the free border of the cell. The nucleus is not differentiated from those of other epithelial cell nuclei.

In the remainder of the tubules of the efferent ducts, the division between initial and terminal zones of the adult is just becoming apparent at this time. The initial zone closer to the testis is made up of epithelial cells whose cytoplasm, here and there in given tubules, appears paler. Such cells are 35 μ high and are beset with a brush border. The cells of the terminal end nearer the canal of the epididymis are about 20 μ tall and the cytoplasm stains darker. The free end often possesses a secretory knob. In both types the lumen is 15–20 μ in diameter and rarely contains a few degenerating spermatocytes, suggesting that some outflow from the testis has commenced.

The epididymal canal shows some degree of differentiation although rather less than the efferent ducts. Three gross anatomical divisions are apparent: head, isthmus, and tail. Within the head there is a configuration of connective tissue septa which permits one to recognize the anlagen of the various zones typical of the adult organ. However, there is no histodifferentiation within these zones as yet. The epithelium shows a similar arrangement of nuclei throughout, although the height of the cell lies about two modes—27 and 22 μ. The lumen increases correspondingly in these two types from 17 to 30 μ (Plate 1, Fig. 5). The portion of the canal with wider lumen occurs apparently at random in the region which is the anlage of zones 3–5 in the adult but does not occur in that region of the head which will develop into the zones of tall epithelium, zones 1 and 2. Thus the tendency to low epithelial type appears some time before there is a corresponding change in other cytological features. The wall of the tubule in the head of the epididymis is composed of a pseudostratified columnar epithelium about 35 μ tall. Compared with the 21-day organ the nucleus is relatively smaller and the cell relatively taller so that nucleocytoplasmic ratios more akin to the definitive adult type now occur. In more caudal regions of the head binucleation of cells is common; a pair of nuclei lie in close apposition, the line of division of the pair being parallel with the surface.

Halo cells are present, lying between individual cells of the epithelium, and they assume many shapes from rounded to trilobed.

In the regions of low epithelium the same pseudostratified appearance and the same nuclear arrangement obtains, the only difference being that the cytoplasm is shorter. This is brought out at the frequent transitions from short to tall epithelia which occur along the length of one segment of canal.

In the tail of the organ at this stage the lumen of the tubule develops a typical star-shaped outline due to the intrusion of triangular-shaped masses of epithelial cells from two or three sites on the periphery. This arrangement, evolved about the 28th day, persists in the tail through to advanced stages of histogenesis. The epithelium is 35 μ at its highest and is composed of tall columnar cells whose basal nuclei are distributed in two to four tiers. This makes for a relatively heavier nuclear concentration than in the head of the organ. The nuclei are elongate and oval and many appear to overlap. Many cells contain two nuclei, one on the lumen side of the other. They are joined by a common nuclear membrane which is straight and thus parallel with the free edge or projected over the sides of the more basal nucleus as a cap. As with the canal in the head of the organ, here and there throughout the length of the canal in the tail are segments of shorter epithelium abruptly alternating with tall epithelium. There is merely a loss in height; the nuclear shape and pattern remains as in the tall areas.

The lumen is devoid of cell content, but in the head many cells have tenuous strands of pink material about their free end. These may well represent the anlagen of stereocilia.

Mitoses are difficult to find in the 4-week-old animal. In one count there were three per 100 tubule cross sections in the canal and none per 100 tubule cross sections in the efferent ducts.

Testis.—There are three well-defined cell types in the seminiferous tubule: peripheral spermatogonia interspersed with occasional comma-shaped cells, and inside these there are two or three rows of primary spermatocytes in the pachytene stage of meiosis.

(*d*) 32 *Days*

Histodifferentiation, apparent in the 28-day epididymis, has advanced by the 32nd day to a stage where unmistakable features of adult-type cells may be recognized.

In the efferent ducts, increased growth has resulted in a prominent region of coni vasculosi about the terminal ends of the ducts. The initial zone is differentiated from the terminal zone by the clarity of the cytoplasm. The difference is, however, at this stage not so well marked as it is some weeks later. Amongst the clear cytoplasm, varying-sized eosinophilic granules and spheres can be discerned much as in the adult. The nuclei have folded margins. Nuclei of ciliated cells are not differentiated from non-ciliated although they tend to assume a position more superficial with respect to the free surface of the epithelial cells than the nuclei of the latter, as in the adult. The average height of these cells is 25 μ. The rete testis has assumed the low cubical epithelium found in the adult.

The terminal portion of the efferent ducts have a cytoplasm slightly more deeply staining. The lumen is filled with spermatocytes and spermatids in what is probably the terminal duct.

In the head of the organ, representatives of all cell types seen in the adult have been differentiated. Zones 1A and 1B are recognized as tall cells up to 40 μ high, i.e. about 60 per cent. of the adult height. The epithelium has the adult content of apical cells and there are long well-developed stereocilia. The connective tissue sheath has been reduced to one or two narrow fibrocyte cells in thickness so that it is now far less conspicuous than at earlier stages.

Zone 1C is shorter (25 μ) but it is recognizably adult-like in the large Golgi zone and the basal position of the nuclei.

Zone 2 is about 23 μ tall at this stage and is differentiated to the extent of possessing apical vacuoles in the cytoplasm. Although these are not as obvious in the cell, and are fewer in number per cell as compared with the adult, nevertheless their presence in even a few cells gives an unmistakable hint to the presumptive fate of this zone. The more uniform row of basal nuclei is also similar to the adult pattern.

Zone 3 is quite small in extent. The average height is 20 μ. The appearance is of a slightly lower continuation of zone 2 in which no cells possess apical vacuoles. However, its origin can be traced from the pseudostratified epithelium present throughout the cranial portion of the canal at the 28-day stage (see below). The Golgi area is already well developed.

Zones 4 and 5 are not yet discrete but at this stage they present four histological types:

(i) A tall epithelium about 45 μ high which is multilayered, having nuclei on approximately two planes toward the cell base. These nuclei are usually oval to round with the long axis perpendicular to the free surface. They are usually open with prominent chromatin networks. One has regarded this epithelium as a persistence of the original pseudostratified tall epithelium present at 28 days.

(ii) A much shorter epithelium about 25 μ high, wherein the nuclear picture is similar to that above but there is little or no cytoplasm in the apical portion of the cell. This is regarded as the first change in the transformation to the low epithelium characteristic of adult stages in these zones.

(iii) An epithelium of similar height to (ii) but wherein the nuclear population, mainly oval, occasionally rounded, has diminished so that the multilayered state is reduced to one layer with occasional interpolated cells resting on the basement membrane. Also the nuclei have become irregular in outline and the long axis of the more oval nuclei is parallel with the free surface so that some apical cytoplasm reappears. The nucleoplasm becomes condensed and binds haematoxylin very strongly. This is to be regarded as nearly the definitive state of the epithelium although, at this stage, the juxta-nuclear vacuoles are not present (Plate 2, Fig. 1).

(iv) More caudally at the constricted portion of the organ and in the tail, the epithelium is again different from the three types above and is more like the epithelium in the analogous areas in the 28-day epididymis. Thus it is tall, up to 75 μ in height, and the free surface is scalloped, so producing a stellate lumen. The nuclei are predominantly rounded to oval. There are no long narrow oval nuclei as are present in group (i) above. Binucleate pairs are very common (Plate 2, Fig. 2).

The arrangements above suggest an extension of the histogenesis of the low epithelium zones 4 and 5 which was first manifest at 28 days. Evidently the first stage is a diminution in height followed by a nuclear differentiation to an irregular-shaped condensed nucleus characteristic of the adult. The process occurs in segments seemingly at random down the length of the canal although it is more advanced at the cephalic end. Isolated low segments are found in the tail.

The lumen throughout the canal is free of cells except for occasional spermatids.

There is no zone 6 at this stage. The tall epithelium of the tail leads into the characteristic thick-walled vas deferens.

The overall mitotic rate in all zones is about eight figures per 100 tubule cross sections.

Testis.—The testis tubules show a heterogeneous content of spermatogonia, pachytene and diplotene spermatocytes, and unattached spermatids in populations evidently relating to the activity of some rhythm (Clermont and Perey 1957).

(*e*) 37 *Days*

There is no qualitative change in this specimen beyond the following:

(i) The initial zone is more sharply demarcated from the terminal zone of the efferent ducts by the increasing supranuclear clarity of the cytoplasm of the former.

(ii) Subapical vacuolation of zone 2 is more extensive.

(iii) Zone 3 is more extensive and appears to be rapidly differentiating from tall epithelium of the pseudostratified type mentioned above. The lumen is full of clumps of spermatocytes and spermatids.

(iv) The mitotic rate at this stage is amongst the highest wherein about one tubule in every three has a mitotic figure. An analysis of the stages in zone 1 showed 50 per cent. of cells in metaphase plate stage with the remainder equally divided between prophase, anaphase, and telophase.

(*f*) 39 *Days*

This was similar to the 37-day specimen except that zone 6 is differentiating from the vas deferens as short cubical cells with pale cytoplasm. Nuclei are oval and round with an open vesicular appearance. They are two to three layers thick. Here and there the even surface of the epithelial cells is interrupted by the extension of a pyriform cell with its apex toward the basal layer and the base rounded and projecting beyond the general surface level. The cytoplasm is highly vacuolated

and it is obvious that these cells, similar morphologically to the "inactive" clear cells of the adult, will develop into "active" clear cells which are a feature of the adult canal in this zone. The lumen contains a large number of spermatids.

(*g*) 56 *Days*

With the first appearance of spermatozoa in the seminiferous tubules, the genitalia can be considered as approaching the mature condition. Progressive changes in the epididymis make it appear more like the adult but histogenesis is still active especially in more caudal regions.

In the efferent ducts the terminal zone has become more adult-like in that the original simple columnar epithelium is becoming stratified and the outline of the lumen is assuming a more folded form. The lumen is still devoid of cell content except in the junctional zone with the epididymis.

Zones 1 and 2 have an adult histological picture and the epithelia have reached their definitive height (60 μ) and lumen diameter (65 μ). Apical vacuolation of zone 2 reaches adult proportions.

Zone 3 shows a large increase in bulk over the 39-day specimen. Its origin from a taller columnar type epithelium is still obvious in one or two tubules toward the periphery of the lobule, otherwise the whole zone is of uniform epithelial height. However, at 30–35 μ tall, it is still higher than the definitive state (Plate 2, Fig. 3).

Active histogenesis is still in progress in zone 4 in that many of the more cephalic tubules have epithelia which are multilayered tall columnar (up to 50 μ tall) (Plate 2, Fig. 4) giving way more posteriorly to an increasing length of tubule with the definitive low epithelium (20–25 μ) containing large numbers of oval and rounded nuclei. A new cytological feature to appear at this stage is the "clear" cells of zone 4. They are conspicuous not so much from the clarity of their cytoplasm which is so obvious in the adult fully developed clear cell, but by the way that their rounded cell base protrudes beyond the free border of the cells on either side. In so doing it interrupts the terminal bar of these cells which by now has become prominent. The deeper end of the clear cell is pointed and so insinuates itself between neighbouring epithelial cells. Its cytoplasm contains large numbers of very small vacuoles and the nucleus is situated toward the outer half. Elsewhere in the epithelium, occasional cells show small vacuoles on the lumen side of the nucleus (Plate 2, Fig. 6). This is the first appearance of the so-called juxtanuclear vacuoles which are a conspicuous cytological feature of zone 4 and 5 in the adult. Occasional clumps of spermatids are present in the lumen of the canal in zone 4.

Zone 5.—Beyond the waist of the organ and in the tail the epithelium is still quite high, up to 70 μ, and multilayered, much as it was when first differentiated at 28 days.

Zone 6.—This zone is similar histologically, although increased in extent as when first apparent at 39 days. By now the clear cells are quite numerous, but still of the inactive type, i.e. conical with the base protruding into the lumen (Plate 2, Fig. 5). The nuclei of the epithelium are of two to three layers, largely round, or bluntly oval but in one or two areas of the periphery of some tubules the epithelium

becomes single layered, an approach toward the definitive condition. The chromatin has no special distribution. Large numbers of spermatids and occasional spermatocytes are present in the lumen. The muscle coat is quite prominent at three to four layers thick. This coat, always well represented about the vas deferens throughout ontogeny, is similarly well developed about zone 6 which appears to be a derivative of the vas deferens.

Testis.—All cell components of the adult testis are now represented from spermatogonia and sertoli cells to elaborated spermatozoa.

(*h*) 72 *Days*

The epididymal ducts now contain a dense mass of sperm in the lumen which at once suggests that an adult stage has been attained. However, histogenesis is still active in the waist and tail of the organ. This is borne out by the mitotic count expressed as mitotic figures per 100 tubule cross sections: zone 1, 6; zone 2, 2; zone 3, 4; zone 4, 19.

The efferent ducts and zones 1, 2, and 3 have an adult appearance. Their heights are of the order found in the adult. In zone 4, one or two tubules at the cephalic end of the zone have a tall multilayered epithelium (up to 50 μ high) as the only evidence of the origin of this zone, the remainder being single-layered low columnar epithelium (about 20 μ) as in the adult. Clear cells are now prominent and careful search shows the presence of juxtanuclear vacuoles amongst the normal epithelial cells.

In the waist, the epithelium is taller (up to 45 μ) and nuclei are elongated in a plane perpendicular to the surface but by now there is one layer only. Juxtanuclear vacuoles are common. The epithelium in the tail also shows a reduction in its multiple-layered nuclei to two or three layers of rounded nuclei. A large proportion of binucleate cells accounts for this two-tiered appearance, the two nuclei lying consistently one above the other.

In the tail, histogenesis in zone 6 has produced a lower epithelium which is still two to three layers of nuclei thick. However, many of the oval nuclei have their long axes parallel to the free surface of the cell. This is apparently the first stage in the development of the adult pattern.

Sperm is present throughout the canal in an adult-like arrangement.

Testis.—The picture in the seminiferous tubules is of the mature testis.

(*i*) 96 *Days*

The predominant histological change is the vast increase in bulk in those zones of wide lumen (zones 4 and 5), so that the head and tail of the organ as a whole increase in volume. Some histogenesis is apparent in the tail region as the mitotic counts per 100 tubules show: efferent ducts, 2; zone 1, 12; zone 2, 5; zone 3, 2; zone 5, 22.

Efferent ducts and zones 1, 2, and 3 are similar to the adult histologically.

Zone 4 has but a short length of tubule with epithelium persisting in the multilayered condition, the vast bulk being of low type. There is evident, however,

gradual nuclear differentiation toward the adult condition. This takes the form of a variation in nuclear size with a range of 2·5 times the diameter from largest to smallest nuclei and of bizarre variations in nuclear shape, the nuclear membrane being wrinkled and thrown up into folds. Clear cells are prominent both of inactive and active type, the latter having a large amount of clear cytoplasm contained in a cell which is columnar rather than pyriform in shape.

Zone 5 in the tail has now an epithelium which is predominantly one layer in thickness although the nuclei are at varying levels; it is truly pseudostratified. The nuclei are mostly oval or elongate and smooth in contour but they show a wide variation in size which is more like the adult condition. Binucleate cells are common but the line of union between the two nuclei has shifted both 45° and 90° from the original which was parallel to the free surface. It is tempting to suggest that this may follow the active growth or passive expansion of the tubule, the nuclei sliding side by side thus converting the original multilayered nuclei into a single layer.

Zone 6 shows further flattening toward the definitive stage of 15–20 μ. The nuclei are single layered in the lowest epithelium and show great size variation. The distribution of chromatin within the nucleus is still central rather than peripheral as it is in the adult.

(*j*) 110 *Days*

Complete maturation to the adult state is evident in this specimen and the following histogenetic changes have brought this about:

(i) In zone 5 the epithelium is low and one cell thick. There is great variety in nuclear size and shape and the more elongated nuclei have their axes parallel with the free surface. There are numerous clear cells.

(ii) In zone 6 the epithelium is one cell thick and the nuclei have become flattened in conformity with the low epithelial height. They also have irregular indentations in the nuclear membranes. The chromatin is peripheral in distribution and apparently adherent to the nuclear membrane.

IV. Discussion

Ontogeny in the epididymal duct follows well-defined stages of development as seen in other organs. Following the determination of the duct, presumably by some effect on the proximal end of the mesonephric duct and associated mesonephric tubules from their proximity to the testis, there follows a comparatively long period of quiescence, at least as far as the evolution of the more obvious cytological details are concerned. Evidently, in response to endocrinological stimuli, there appears a relatively enormous increase in the mitotic rate about the 30th–32nd day resulting in the rapid elaboration of a histological pattern, which, having regard to its complexity, is not far removed from the definitive state. Following this rapid phase, the resumption of a more leisurely mitotic rate produces somewhat slower changes particularly in cytodifferentiation which, over the ensuing 11 weeks, results in the elaboration of the fully differentiated adult state.

During this study of development, a few observations have emerged bearing on the wider problem of differentiation. The limitations of studying dynamic processes by fixed stages have to be constantly borne in mind. First the differing time relations of differentiation along these ducts is noteworthy. Differentiation of the efferent ducts is particularly precocious for they can be distinguished from the remainder of the canal at 3 days, largely by the quality of the connective tissue investment (see below). A little later, in the head of the canal itself, variations in the connective tissue sheath become obvious when the lining epithelium is, as yet, morphologically undifferentiated. It may be suggested in regard to the efferent duct precocity, that the mere prior usage of efferent ducts as functional mesonephric tubules, as opposed to the purely conduit function of the mesonephric duct which they join, would be sufficient to ensure that the cells of the former undertook a different histogenetic path almost from their appearance. The precocity in the histogenesis of the efferent ducts in man was also evident from Pfeiffer's studies.

The precocity of the ciliated cells of the efferent ducts as the earliest appearance of fully differentiated cells seems general for a wide variety of mammals. In fact, their appearance in the rat is relatively late compared with Benoit's (1926) description of their presence in the foetus of ruminants. The other precociously developing segment is the deferent duct which has its definitive histological structure elaborated by the 7th week. Within the epididymal canal itself it is noteworthy that the development of the zones with tall epithelium in the cephalic portion of the organ precedes the development of those with short epithelium in the caudal portion. Indeed, as late as the 96th day, at least, 21 days after the appearance of spermatozoa in the lumen of the canal, histogenesis is still active in the most caudal zones.

It is difficult to hazard a suggestion as to the basis of the differing time relations in histogenesis particularly in the absence of any knowledge of the function or comparative histology of the various zones. Pfeiffer suggests the action of the male sex hormone and of the fluid flow along the ducts in bringing about differentiation. Whilst this is undoubtedly true of why it occurs it does not assist in explaining how. The picture is equally well described by postulating the presence of a gradient field with maximal activity at the testis tapering off toward the caudal end of the duct.

It appears from this study that as a result of activity in the testis, a flow of fluid from that organ is established very early, as witness the presence of spermatocytes and spermatids in the lumen at the 32nd day. Thus the mechanism for sperm transport is already instituted so that when spermatogenesis is sufficiently advanced and free sperms are produced in sufficient numbers they merely exploit the extant flow. In clarification of the requirement for sufficient numbers of spermatozoa it may be noted that although all stages of spermatogenesis are represented in the 56-day animal, including the gametes themselves, these do not appear in the epididymis until some 10 days later. When they do appear, however, they already lie in an arrangement within the lumen specific for the various zones as in the adult.

Although factors underlying the histogenesis of the various zones is obscure, it may be noted that the increase in the lumen of those zones, which in the definitive state possess a wide lumen (e.g. zones 4, 5, and 6), occurs at foci here and there down the length of the tube. Presumably the entire tube is dilated when, during the

progression of the effect, neighbouring foci come to overlap. This mechanism is seen in other tubes during embryogenesis, e.g. gut. Benoit (1926) speculated that the activity of the sex hormones may be mediated by foci in the area of blood vessel distribution. The effect under discussion might well be an illustration of Benoit's concept.

As far as can be deduced from these observations, the process of widening of the lumen is associated with a concomitant nuclear differentiation. The multilayered nuclei of the early stages appear to slide over each other and to change their long axes through 45° to a final plane of 90° from the original, so that this axis now parallels the free surface of the cell. The cell itself widens and the duct lumen is enlarged accordingly. At the same time the chromatin material becomes more dense and features characteristic of the definitive state of the nucleus appear, e.g. folding of the nuclear membrane. Thus the whole process of duct enlargement could be explained by any factor which brought about cell differentiation *per se*.

Concerning the changes in nuclei during histogenesis of these ducts, three observations are pertinent. Firstly, where nuclear differentiation is manifest, e.g. by folding of the nuclear membrane or increasing density of chromatin material, it accompanies the transition to the definitive state. Thus in zones 4 and 5 during the multilayered phase of nuclei in the epithelium, the nuclei are uniformly open-textured with smooth contours. As the epithelium becomes more simple and lower in height so does the nuclear picture change to the irregular outlines characteristic of the nuclei of the adult in these zones. Secondly, the appearance of binucleation is very common in all zones, especially caudally in the organ, and is relatively early in onset, i.e. at 32–37 days. The origin of the pairs is obscure. They may occur at all levels from superficial to deep in the multilayered nuclei of caudal zone epithelia usually one above the other. When the epithelium becomes lower during histogenesis the members of the pair lie side by side. This distribution does not conform to that of the axis of mitotic spindles of dividing cells in the epithelia of various zones of these ducts which has been observed to take up variable directions with respect to the free surface of the cell. Lastly, Benoit's observations of the decreasing nucleocytoplasmic ratio during histogenesis is apparent. In the low columnar cells of the duct of the 3- and 21-day specimens the nucleus occupies up to three-quarters of the volume of the cell. There is a continuous increase in the relative bulk of cytoplasm thence onwards.

The appearance of variable quantity and histological appearance of the cells of the connective tissue investment to the different parts of the developing epididymal ducts may be of significance in the light of the work by Grobstein (1953) who showed a differential response on the part of developing mouse salivary gland tubules to the mesoderm with which they were experimentally cultured.

Clear cells of the type referred to in Part I (Reid and Cleland 1957) as "inactive" clear cells appear in the 56-day epididymis after the epithelium has reached its definitive height. It appears that their apparent inactivity may be due to their embryonic state. "Active" clear cells are not apparent until the 72-day specimen when sperm is found in the lumen.

Obvious defects in the method of recording the mitotic rates in these observations are the difficulty of striking a standard in a growing tissue and the discontinuity in the time of recording, i.e. there may have been other peaks undetected between the age groups used here. However, the trend was similar for both animals examined and it may be valid to suggest that there are two peaks for the organ as a whole, at 3 days and at 37 days. The latter is undoubtedly associated with the maximal period of histogenesis. The rate settles down to the adult one (*c.* five per 100 tubule cross sections) in the more cranial extent of the duct by 56 days but does not simulate the adult rate in the caudal zone, where histogenesis is delayed by comparison until 110 days. Again the overall control of mitotic rate is undoubtedly endocrinal in origin with the characteristic delicate correlation between the maturation of the spermatozoa and of the ducts to receive them.

V. Acknowledgment

The work has been aided by grants from the University of Sydney Cancer Research Fund.

VI. References

Benoit, J. (1926).—Recherches anatomiques, cytologiques et histophysiologiques sur les voies excrétrices du testicules chez les mammifères. *Arch. Anat., Strasbourg* **5**: 174–412.

Clermont, Y., and Perey, B. (1957).—Quantitative study of the cell population of the seminiferous tubules in immature rats. *Amer. J. Anat.* **100**: 241–67.

Grobstein, C. (1953).—Epithelio-mesenchymal specificity in the morphogenesis of mouse submandibular rudiments *in vitro*. *J. Exp. Zool.* **124**: 383–403.

Ludford, R. J. (1925).—Some modifications of the osmic acid methods in cytological technique. *J. Roy. Micr. Soc.* **1925**: 31–6.

Pfeiffer, E. (1928).—Die Entwicklung der keimleitenden Wege des Mannes. *Z. mikr.-anat. Forsch.* **15**: 472–598.

Reid, B. L., and Cleland, K. W. (1957).—The structure and function of the epididymis. I. The histology of the rat epididymis. *Aust. J. Zool.* **5**: 223–46.

Tao, T. S., and Eaton, O. N. (1954).—Post-natal growth and histological development of reproductive organs in male goats. *Amer. J. Anat.* **95**: 401–20.

Explanation of Plates 1 and 2

Epididymal ducts of young rats were fixed in Helly's fluid to which formalin and acetic acid were added. Figures are selected from serial sections at 8 μ thickness stained with iron haematoxylin and eosin. $\times c.$ 1000

Plate 1

Fig. 1.—3-day efferent duct. The epithelium is cuboidal and the nucleus occupies over half the volume of cytoplasm. The connective tissue investment is relatively thin and the fibrocytes have elongated nuclei.

Fig. 2.—3-day epididymal duct. The epithelium is similar to that in the efferent ducts. Mitotic figures are common at this stage and one is shown. The connective tissue investment is thicker and the fibrocyte nuclei are plumper.

Fig. 3.—21-day efferent duct. The epithelium is still undifferentiated and the connective tissue investment is tenuous

Fig. 4.—21-day epididymal duct. Undifferentiated epithelium surrounded by a thicker coat of connective tissue for contrast with Plate 1, Figure 3.

Fig. 5.—28-day epididymal duct from the caudal portion of the head. Portions of three tubules are shown. That on the right shows tall pseudostratified columnar epithelium. The central tubule shows the decrease in cell height and some simplification of the epithelium which occurs in foci in the duct in this region. That on the left shows, in the same cross section, a transition between the two former epithelial types.

Plate 2

Fig. 1.—32-day epididymal duct from the caudal portion of the head. A further stage in the histogenesis of the low epithelium portion pictured in the central tubule in Plate 1, Figure 5. Early nuclear differentiation is manifest by notching of the nuclear membrane evident in one cell about 8 o'clock. Halo cells are frequent and a mitotic figure is present at 1 o'clock. Mitoses are frequent at this age. The free edge of the cells bear stereocilia and the lumen contains several spermatids.

Fig. 2.—32-day portion of the wall of the epididymal duct in the tail of the organ. Nuclei are in many rows and are predominantly oval in shape with the long axis perpendicular to the free surface. The lumen is stellate and is produced by three or four narrow areas about the periphery wherein the cells are shorter.

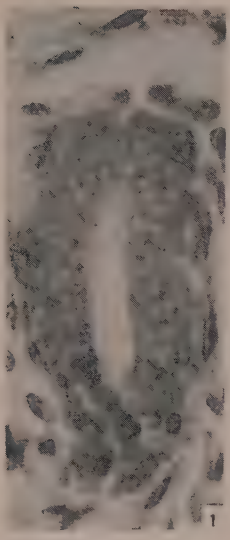
Fig. 3.—56-day portion of the wall of two tubules in the head of the organ in presumptive zone 3. A clear area is present in the apical cytoplasm but it is smaller than in the mature cells of this zone. Stereocilia are also present but there are no spermatozoa in the lumen.

Fig. 4.—56-day portion of the wall of the duct towards the cranial end of presumptive zone 4 representing the residue of the tall multilayered nuclei epithelium whence the definitive zone 4 is derived. A later stage of differentiation of this epithelium is shown in Plate 2, Figure 6.

Fig. 5.—56-day portion of the wall of the duct in presumptive zone 6 in the tail. The epithelium still has two or more rows of nuclei and the muscle coat, occupying all of the figure beneath the epithelium, is thick. Two inactive clear cells are shown with vacuolated cytoplasm. Towards the right of the figure a spermatid lies on the free surface.

Fig. 6.—56-day portion of the wall of the duct, in zone 4, from a tubule nearby that in Plate 2, Figure 4. The reduced height and simplification of the epithelium is apparent. Some of the cells show vacuolation equivalent to the juxtanuclear vacuoles of zone 4 in the definitive state. Binucleate figures such as that at the extreme right of the picture are common in all developing, as well as adult, zones.

THE STRUCTURE AND FUNCTION OF THE EPIDIDYMIS. II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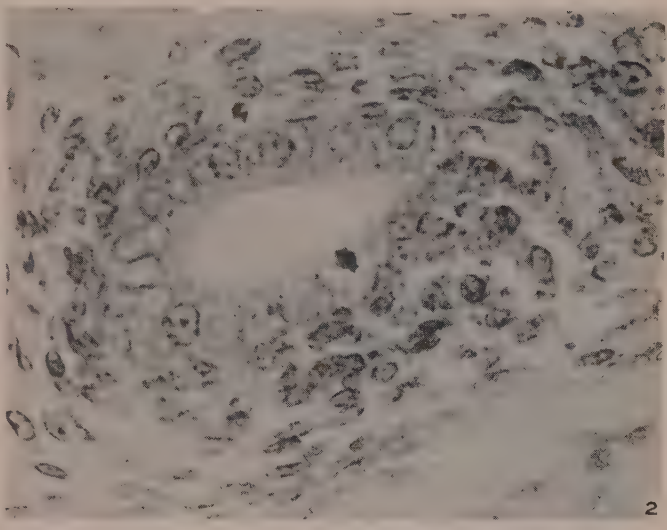
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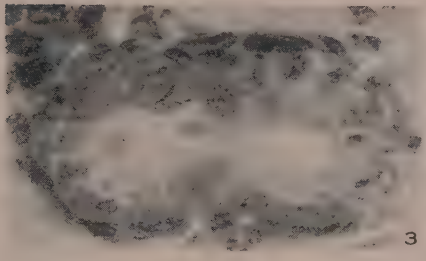
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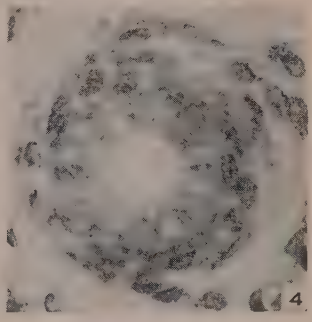
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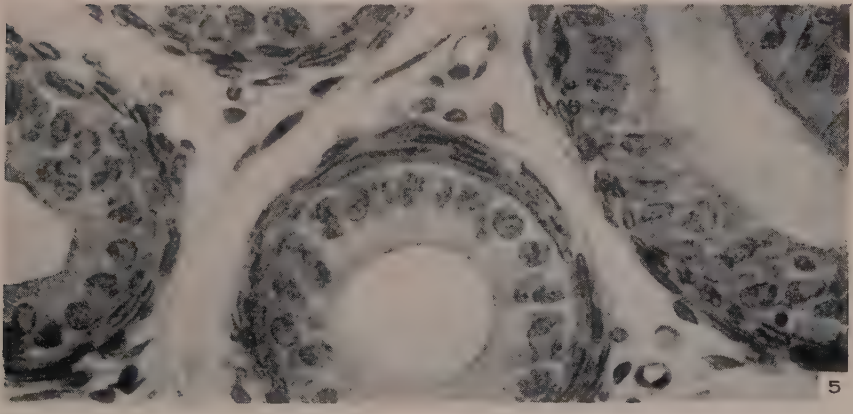
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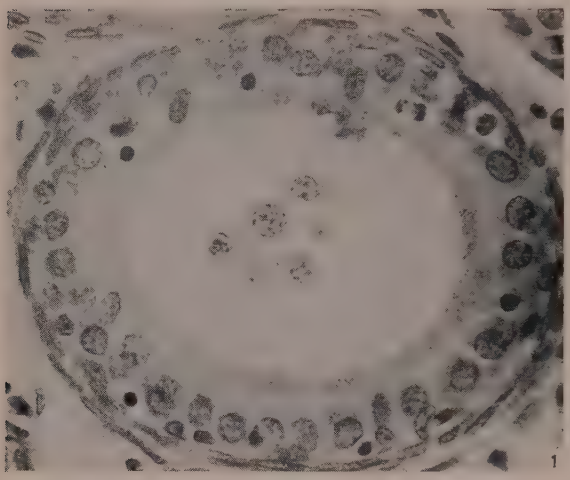
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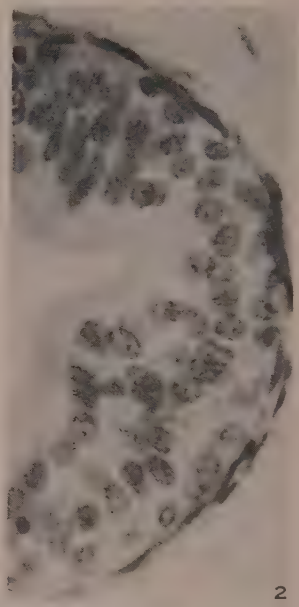
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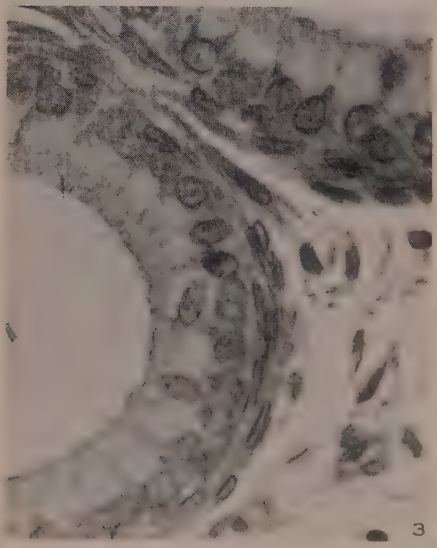
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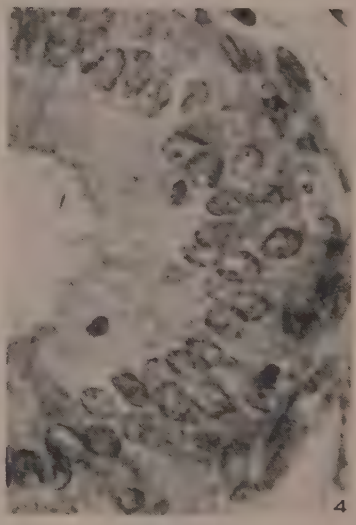
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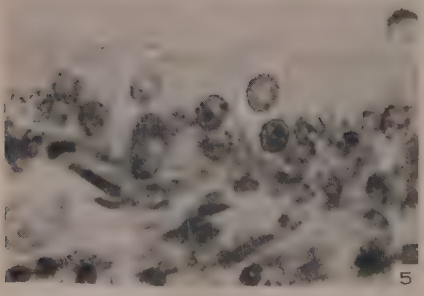
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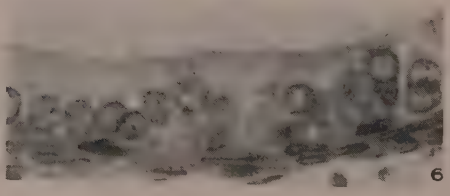
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OBSERVATIONS ON THE ECOLOGY OF THE CLOTHES MOTHS *TINEOLA BISSELLIELLA* (HUMM.) AND *TINEA PELLIONELLA* L. IN A BULK WOOL STORE

By K. H. L. Key* and I. F. B. Common*

[*Manuscript received November* 18, 1958]

Contents

Summary

Observations on the behaviour and abundance of *Tineola bisselliella* and *Tinea pellionella* were made during 1942 and 1943 in a Brisbane wool store which remained relatively undisturbed for more than three years. The store contained *c.* 10,000 strongly compressed, jute-covered "double dumps" of low-grade, greasy wool, stacked in large bays separated by passages. Microclimatic conditions were more equable than in the open and very favourable to the moths. Substantial vertical gradients in temperature and relative humidity resulted from insolation of the roof, but there was little horizontal differentiation. The wool tended to buffer fluctuations of these elements, both in the free air spaces and, still more, within the dumps.

Larvae of *Tinea* were confined to loose wool protruding through breaches in the jute pack. *Tineola* occurred both there and within the surface 2 in. of the

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compressed wool under the pack. By 1943 there was estimated to be an average of *c.* 50 well-grown larvae per dump. Adult moths could always be seen sitting or running on the dumps throughout the stacks.

The moth populations were studied by sampling with tanglefoot traps in the passages and stacks. Both species had a flight period around dusk, about half the 24-hr catch being made during the 3 hr after sunset. The peak fell earlier on cool evenings. A regression of activity on mean flight period temperature was established, a rise from 65 to 80°F doubling the catch. Females rarely fly: they normally constituted only *c.* 0·3 per cent. of the catch, although the sex ratio in the population as a whole was probably about 2♂ : 1♀ for *Tineola* and 1♂ : 4♀ for *Tinea*. The density of moths in flight was approximately equal in stacks and passages at a given level, but usually increased with height.

By correcting the daily catches for the effect of temperature on activity, plots of adult abundance against time were constructed. The two species fluctuated in almost identical fashion. Numbers were low in winter, high in spring and summer, with an indication of two to three generations per annum. From the beginning of 1943 a great decline in abundance set in, the peak for that year being a small fraction of that for 1942.

The braconid *Apanteles carpatus*, a parasite of both moths, was also taken on the traps. It had a flight period at about the time of the daily temperature maximum. A regression of activity on temperature showed a near-quadrupling of the catch for a rise from 70 to 85°F. A plot of adult abundance was derived as for the moths. It showed a single seasonal peak falling after the moth peaks. Several overlapping generations are postulated. The abundance ratio of *Apanteles* to moths rose steadily from spring to autumn in each year, but showed close agreement for corresponding seasons of the two years: there is no reason to ascribe the 1943 decline in moth numbers to the parasite.

A spider, *Uloborus geniculatus*, became very abundant during 1943 and is believed to have been responsible for the decline of the moths, which represented its main source of food. Over the relevant period the percentage of female moths in the catch increased 40-fold, presumably as a result of the differential removal of the active males by the spider. It is calculated that in this way the number of males was reduced, by November 1943, to about 1/45 of what it would otherwise have been. The spider probably also caught adult *Apanteles*.

It is concluded that, apart from effects of the introduction of additional species, the moths and spiders would ultimately establish some sort of equilibrium, probably at a rather low level of density. The factors favouring *Uloborus* in its limiting role are discussed.

I. Introduction

During the second world war, baled wool was held in store in Australia for very much longer periods than normal. Heavy infestations of the cosmopolitan clothes moths *Tineola bisselliella* (Humm.)* and *Tinea pellionella* L.* developed in most of the wool stores in Brisbane and Sydney and to a less extent in other ports, and the Commonwealth Scientific and Industrial Research Organization was asked to undertake investigations with a view to control. As one aspect of these, a study was made of the behaviour, distribution, and population changes of the two species in a Brisbane store where most of the wool remained undisturbed for more than three years.

*Identification by I. F. B. Common.

The store chosen was the Queensland State Wool Committee's "C" store, situated at New Farm Wharf. It contained low-grade wool from the 1939–40, 1940–41, and 1941–42 clips, the bulk of it 1940–41. Most of this wool had entered the store in the closing months of 1940, the remainder in October and November 1941. Observations were begun in December 1941 and continued until March 1944.

II. Environment

(*a*) *Construction of the Store*

The store was constructed in 1940 on previously vacant land. It was a lightly built, single-storey structure, about 220 by 120 ft and not subdivided. It had a brick foundation wall extending about 4 ft above ground; the hardwood floor was carried on this and numerous brick piers of similar height. The walls were about 20 ft high and consisted of overlapping hardwood weatherboards. The roof sloped at a slight angle from a longitudinal central ridge 29 ft above floor level. It was covered with corrugated asbestos-cement sheeting and supported by numerous hardwood posts. Only one of eight sliding doors, 10 by 9 ft 6 in., was ever opened during the period of the study. This was at the front end of the store, facing NNW.; it was kept closed when not in actual use. Twenty-six windows, each 4 by 6 ft, were located high up along the walls; they were kept permanently closed.

(*b*) *The Wool*

Almost all the wool in C store was low-grade greasy Merino (skirtings, locks, crutchings, skin wool, belly wool, pieces, etc.), much of it soiled in varying degree with urine, faeces, or blood, and containing burrs. In common with all wool prepared for shipment during the war, it had been "dumped". Wool from the country reached the ports in "bales", under light compression within a jute pack. After appraisement, bales containing wool of the same type were placed head to head in pairs and compressed under 800 lb/sq. in. The resulting "double dump" was secured by four steel wires or bands (Plate 1, Fig. 1); double dumps varied in weight from about 400 to 1100 lb and also in size.

In the course of appraisement a proportion of all bales was opened by a cut or "porthole", some 12 in. or more in length, near a corner of the bale. This was roughly sewn up afterwards, but a considerable area of wool remained exposed. At dumping, the pack often gave way at the sites of these portholes and at other points of weakness, with the result that a mass of wool some inches in diameter protruded from the dump (Plate 1, Fig. 1; Plate 3, Fig. 1). Breaks also occurred to some extent later, in the stacks. Their characteristic position was along the seam at the corners of the heads of the bales, which in the double dump are pressed together along its median transverse plane, but they also occurred elsewhere. In C store the average number of large breaks 3 in. or more in diameter was about two or three per bale and few bales were without them. In addition to these large breaks, every dump had a variable number of small tears and holes, $\frac{1}{8}$–$\frac{1}{4}$ in. in diameter, produced by the wool hooks used in handling the dumps; each of these exposed a small area of wool.

As a consequence of the method of dumping and wiring, each dump had three types of face differing in surface features and in the stress experienced over the

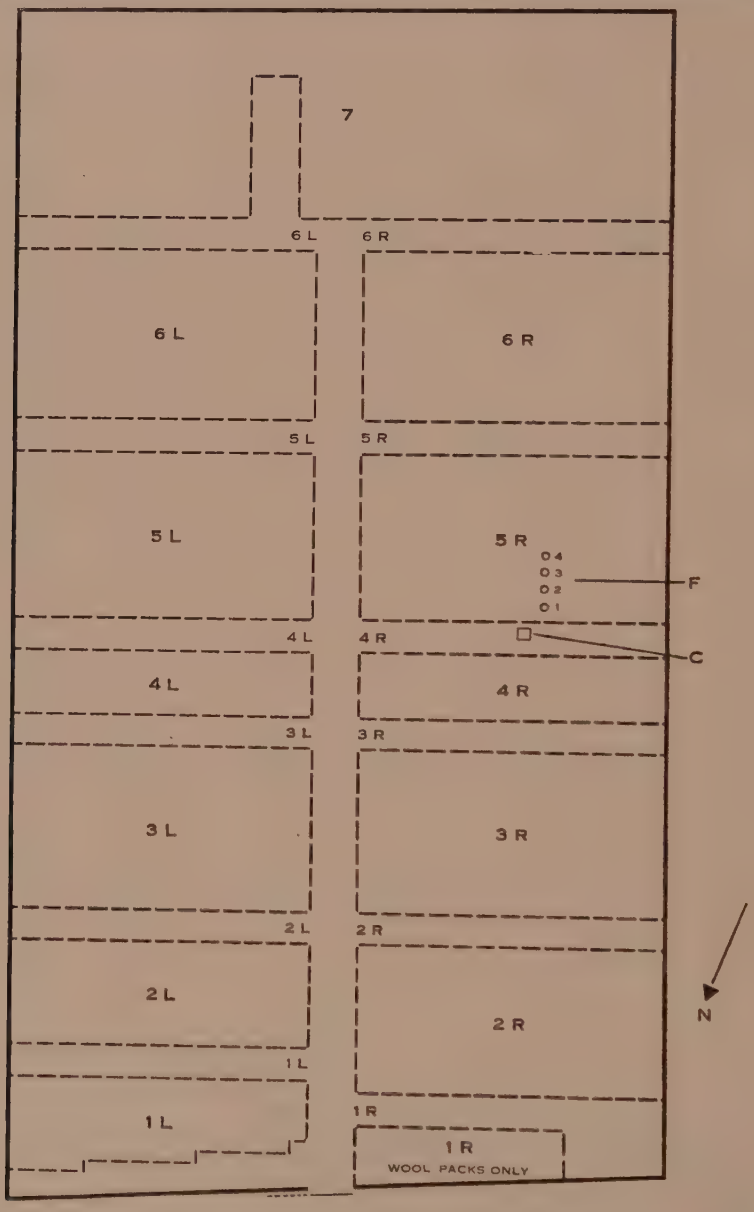

Fig. 1.—Sketch plan of C store, showing disposition of wool stacks and passages at the commencement of the investigations. *C*, cubical trap; *F*, row of four funnels in which trapping was carried out in stack 5R.

face; these may be designated I–III (Plate 1, Fig. 1). Face I comprises the two ends of the dump, or the bottoms of the constituent bales. It was strongly convex,

very taut, deeply indented by the wires, and unwrinkled. Face II is the lateral face traversed longitudinally by the wires (which made only slight indentations except at the junction with face I) and transversely by folds in the pack and wool due to the compression of the bales in the direction of their long axes. Face III is the other lateral face, not traversed by wires, but likewise thrown into transverse folds. Although there is no direct evidence, it seems that the degree of compression of the wool would have been greatest over I, less over II, and least over III.

Within C store the dumps were stacked in solid blocks (Fig. 1). From the front door a "main passage" about 9 ft wide ran down the central axis of the store. The stacks were arranged on each side of this, separated by "alleys" about 6 ft wide set at right angles to the main passage, and reached to within some inches of the walls of the store. The dumps were stacked vertically on top of each other to a height of six (sometimes five) dumps, i.e. about 15 ft; 16–17 dumps went to the length of the stack at right angles to the main passage (*c.* 55 ft) and 5–10 to its width parallel to the passage (average *c.* 30 ft). The various tiers of dumps were separated by dunnage, which served to hold the stack together. The dumps were given a consistent orientation within the stack, such that face I was vertical and parallel to the main passage, II vertical and parallel to the alleys, and III horizontal (Plate 1, Fig. 2; Plate 3, Fig. 1). Air (and moths) could circulate freely through the stacks, particularly along the horizontal "tunnels" and the wider vertical "funnels", 6–12 in. in diameter, left between the edges of four adjacent dumps on account of their curvature.

Each stack usually included several different "invoices". The dumps of each invoice were stacked together, but were not separated in any way from those of other invoices. Thus a given stack could contain wool of different age, type, and period of retention in store—hence also of different degree of infestation by moth. Any movements of wool were by whole invoices. The small amount of outward (mainly) or inward movement that took place thus involved partial dismantlement and subsequent rebuilding of the stacks affected.

The store contained in all some 10,000 dumps, representing several million pounds of wool.

(*c*) *Microclimate*

Information on the microclimate of the store was obtained from measurements of temperature and humidity at various points in the passages and of temperature in the interior of a stack and at a depth of 2 in. within a dump. A few measurements of light intensity were also made. Air movement within the store was negligible.

(i) *Temperature and Humidity in the Passages*

From April 21, 1942, until May 27, 1943 (with gaps of a few days), and again for portions of the months October–December 1943, a thermohygrograph was operated at the cubical trap to be described later (Section IV(*a*)). It was located in alley 4R (Fig. 1), midway between the floor and the top of the adjacent six-tier stack. Before this, temperature readings had been taken at 3-hr intervals over the period April 10–13, 1942, by means of mercury-in-glass thermometers suspended at about 2 in. from three faces of the trap.

Over the same period and at the same 3-hr intervals, readings of temperature and relative humidity, the latter by means of Edney paper hygrometers, were

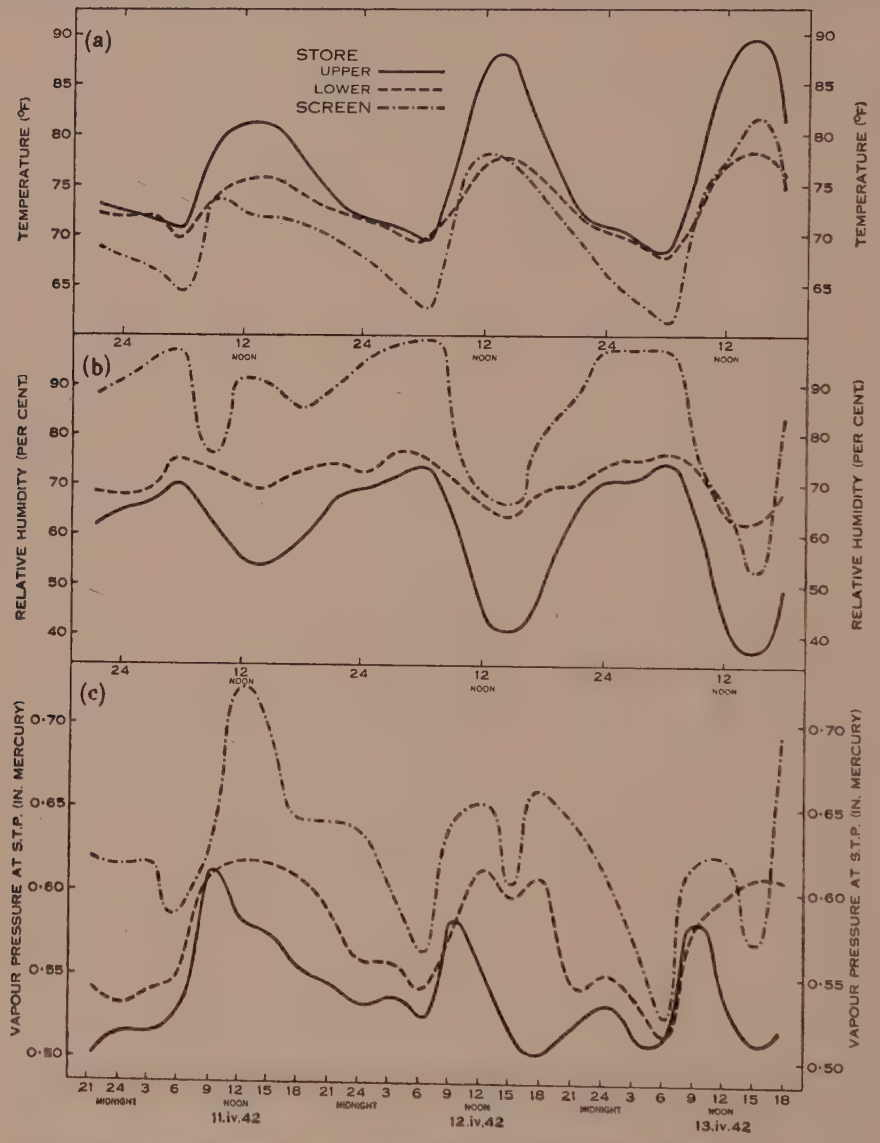

Fig. 2.—Smoothed curves for temperature (*a*), relative humidity (*b*), and vapour pressure reduced to S.T.P. (*c*), based on approximately three-hourly temperature and relative humidity readings at the levels of the highest ("upper") and lowest ("lower") dump of the stacks at the intersection of the main passage and alley 4, and on readings for the corresponding times at the Brisbane climatological station ("screen"). April 1942.

taken at the east end, west end, and centre of alley 4 and at the centre of alley 6 (Fig. 1). The instruments were suspended on strings, one set being level with the mid-

point of the lowest dump of the stacks at each location and another with that of the highest (sixth) dump. A similar series of readings was taken over the period July 24–26, 1942, at the east end, west end, and centre of alley 3 and at the centre of alleys 1 and 5. In this way a comprehensive picture was obtained of the vertical and horizontal temperature and humidity differences in the lower 15 ft of the store occupied by the wool.

(1) *Temperature.*—It may be seen from Figures 2(*a*) and 3 that the diurnal temperature regime for both the upper and lower positions in the store was broadly similar to that at the Brisbane climatological station. However, the curves for the upper position show considerably higher maxima than those for the lower. The means for all the upper readings in July do not differ as between the different sampling points by more than 0·5°F, or those for the lower readings by more than 1·1°F. Gaps in the April series of readings prevent the calculation of means, but the picture is much the same. Clearly the horizontal temperature differences within the store can be of no biological significance, while the vertical differences could be.

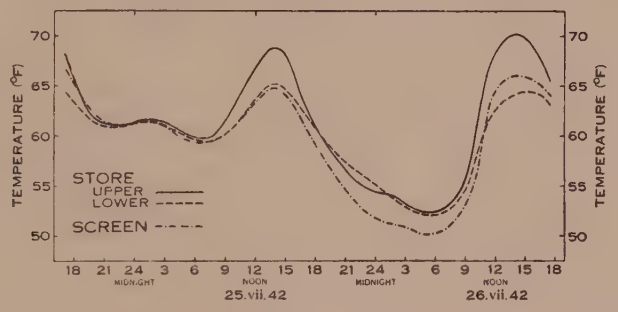

Fig. 3.—Smoothed temperature curves based on the means of approximately three-hourly readings at the levels of the highest ("upper") and lowest ("lower") dump of the stacks at five points in the passages, and on readings for the corresponding times at the Brisbane climatological station ("screen"). July 1942.

The temperature at the cubical trap in alley 4R was very close to the mean of the values for the upper and lower positions at all sampling points; it may thus be used as a satisfactory single measure of the temperature in the passages. An approximately linear temperature gradient between the floor and the top of the stacks is also indicated.

The maximum temperature determined from the thermohygrograph in the store was somewhat lower than that in the screen for most months of the period May 1942 to April 1943 (Fig. 4). The minimum was considerably higher in the store in every month. Thus the temperature in the passages was on the whole more equable than that outside, with a higher mean value, although on clear days the store maximum tended to be higher than the screen, as a result of radiative heating from above. The seasonal progression of temperature closely paralleled that in the screen. The overall mean for the period covered by Figure 4 is 71·7°F.

The lowest daily minimum given by the store thermohygrograph over the period of the observations was 50·5°F (Fig. 4). In view of the absence of any

appreciable vertical temperature gradient at the time of the minimum (Figs. 2(*a*) and 3), we may consider a temperature of about 50°F to be the lowest attained anywhere in the store. A conservative estimate of the highest temperature attained within the passages up to the top of the stacks may be obtained by adding to the highest daily maximum given by the thermohygrograph a quantity equal to half the maximum temperature difference observed between the upper and lower positions (14·7°F). This gives a figure of about 97°F, which is probably an underestimate.

(2) *Humidity.*—If the relative humidity curves of Figure 2(*b*) are compared with the corresponding temperature curves (Fig. 2(*a*)), it may be seen that each humidity curve resembles an inversion of the corresponding temperature curve;

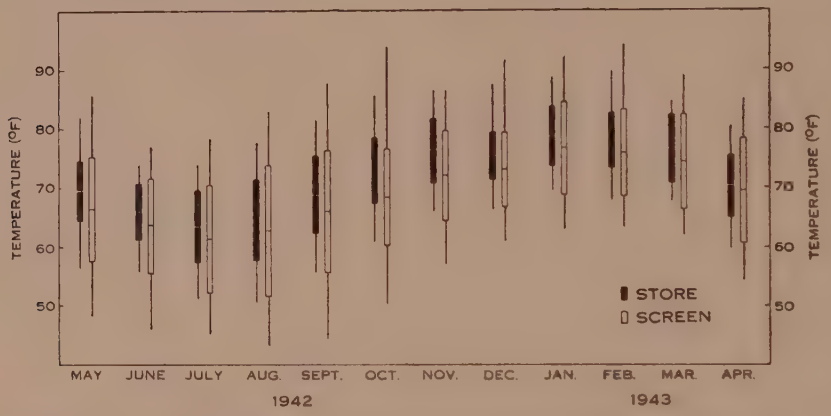

Fig. 4.—Monthly mean maximum and minimum temperature, with corresponding extreme maxima and minima, as given by the thermohygrograph in C store and the records of the Brisbane climatological station, for the period May 1942 to April 1943. The mean values are given by the ends of the broad columns and the extremes by the ends of their narrower extensions. Values for November, December, and April based on incomplete data, but comparable as between store and screen.

moreover, each of the store humidity curves is more similar to the corresponding inverted temperature curve than to the screen humidity curve. It follows that the differences in relative humidity between the upper and lower positions, as well as the diurnal relative humidity regime, are determined mainly by the temperature differences. In conformity with this relation, we find that the humidity curve for the lower position is higher throughout than that for the upper, the differences being small at the maxima but considerable at the minima. On the other hand, horizontal differences between readings at different sampling points were slight.

Comparison of hygrograph traces from store and screen shows again that, although there is a general correspondence in their fluctuations, this appears to be due more to the general dependence of relative humidity on temperature in both situations than to any direct connexion, for during several periods when the outside fluctuations were abnormal, those in the store were hardly affected. In general, the range of fluctuation in the store was smaller than in the screen, mainly because the store minima were considerably higher.

Comparison of Figure 2(*c*) with 2(*a*) shows that the vapour pressure curve (calculated to S.T.P.) for each situation is broadly similar to the corresponding temperature curve. Although the curves show some unexplained features, it is clear that, in all three situations, water vapour is continually being added to the air as the saturation deficit rises with rising temperature and removed from it as the temperature falls. The relationship is closer in the store than in the open. It is obvious that the long-period mean vapour pressure must be the same in the two situations and that the hygroscopic wool and wool packs must come into equilibrium with that value. However, the resistance of the store to movement of air, and the buffering effect of the wool, result in the period required for equilibration being so much longer than that in which wide variations are possible in the outside vapour pressure that the store humidity at any moment can be regarded as largely independent of that outside.

TABLE 1

LATE AFTERNOON TEMPERATURES (°F) AT THE TOP OF THE FIRST TO FOURTH AND SIXTH TIERS OF DUMPS IN STACK 5R, ON TOP OF A CROSS-BEAM ABOUT 4 FT ABOVE THE STACK, AND AT A DEPTH OF 2 IN. IN A DUMP OF THE SIXTH TIER

Date	First Tier	Second Tier	Third Tier	Fourth Tier	Sixth Tier	Cross-beam	2 in. in Dump
16.ii.42	76	78	79	80	90	—	—
19.ii.42	76	78	79	80	80	—	82
23.ii.42	78	80	82	84	99	101	88
26.ii.42	77	79	80	82	92	94	86

(ii) *Temperature within and above the Stacks and in the Surface Layers of the Bales*

A few data on stack and bale temperatures were obtained from readings taken at different depths in one of the vertical funnels, on a cross-beam about 4 ft above the top of the stack (about 6 ft from the roof), and within a dump of the sixth tier at a depth of about 2 in. below its upper surface. The striking feature of the temperature distribution (Table 1) is the gradual rise within the stack, followed by a sudden "jump" to the sixth-tier reading and then a further gradual rise to the cross-beam. Since the reading for the sixth tier corresponds to the top of the stack, the wool appears to have sharply divided the air of the store into a relatively cooler layer in the stacks and a warmer layer above them, the temperature in both rising slowly with height. On February 19, 1942, the differentiation was lost, presumably because this day was overcast with intermittent rain. The temperature within the dump shows even more clearly the stabilizing influence of the wool. This effect must be important in utilizing the heat from the roof to maintain a temperature suitable for rapid multiplication of the moths, whilst hindering the development of temperatures that would have harmful effects.

Figures for an experimental stack in the south-eastern corner of the store (Table 2) are similar to those of Table 1, except that the difference between the temperatures at the top of the stack and those lower down is less. The temperature at the third tier is very close to that given by the thermograph situated at the same height in alley 4 but shows the buffering that would be expected. The mean over the 4 days may be regarded as identical in stack and passage. Since in the passages the temperature at half the height of a stack is equal to the mean of the values for the top and bottom tiers, we may regard the thermograph readings as representative not only of the temperature of the passages, but also of that within the stacks, although at times of rapid temperature change the figure for the stacks would show a discrepancy in the conservative direction.

TABLE 2

MORNING TEMPERATURES (°F) AT THE TOP OF THE SECOND, THIRD, AND SIXTH TIERS OF DUMPS IN AN EXPERIMENTAL STACK, WITH THE CORRESPONDING READINGS GIVEN BY THE THERMOGRAPH IN ALLEY 4

Date	Second Tier	Third Tier	Sixth Tier	Thermograph
5.iii.43	77·4	77·9	82·9	79·5
6.iii.43	76·8	77·0	79·9	76·6
7.iii.43	73·0	73·6	75·4	72·4
8.iii.43	72·3	73·0	74·5	71·6

(iii) *Light Intensity*

Measurements of light intensity, using a Weston exposure-meter, were made at the site of the cubical trap in the forenoon of 10 days in April and May, 1942. The instrument was directed towards the faces of each of the flanking stacks, up and down the alley, and vertically upwards and downwards. Only two of these six readings ever exceeded 1 ft-candle, namely that with the instrument directed towards the west wall, which had a window directly facing the alley (5–28 ft-candles, mean 17·8), and that with it directed towards the roof (1–4·5 ft-candles, mean 2·6). Intensities below 1 ft-candle could not be accurately measured with the instrument available. If all readings of less than 1 ft-candle are given the value of 1·0, we get a mean for all readings of 4·1 ft-candles.

At times shafts of direct sunlight entered the store through one or other of the windows, giving rise to local patches of high light intensity. Although this did not happen during the morning at the site of the cubical trap, the latter was certainly located in one of the better lit parts of the store, so that the average daytime light intensity in the passages must have been well below 4 ft-candles for the store as a whole. Very much lower values must have obtained within the stacks.

At night the store was quite dark, except on the rare occasions when the widely spaced electric lights were used.

(*d*) *Other Animals in the Store*

Lyctus sp. was present in the dunnage of the stacks. Mosquitoes were very numerous. Three species of small parasitic Hymenoptera were taken from time to time, sometimes in quite large numbers, on the tanglefoot traps used for sampling the moth population. The commonest of these was *Apanteles carpatus* (Say),* a well-known braconid parasite of both species of clothes moth (Fallis 1942). Cocoons of this species were found inside cocoons of *Tineola* in the store and the adult parasites were bred from laboratory stocks of both species of moth obtained from the Brisbane wool stores. Further information on the behaviour and abundance of *A. carpatus* in relation to the host populations will be given in later sections. The other two Hymenoptera were *Chremylus rubiginosus* Hal.,* known as a parasite of *Tinea pellionella*, and *Doryctes* sp.,* probably a parasite of the *Lyctus* sp.

At the beginning of the investigation, spiders were present in the stacks in small numbers, chiefly within about 10 ft of the walls; by December 1943 the numbers had increased enormously. Apart from a few Pholcidae occasionally seen at the base of the stacks and a few funnel-builders usually associated with posts or with the wooden walls, the population consisted of the well-known holotropical species *Uloborus geniculatus* (Olivier).† This extended throughout the stacks, but was commonest towards the outside, i.e. near the passages. It built orb webs in the funnels and tunnels, which by early 1944 were densely festooned with webbing (Plate 2). In the gaps between the dumps facing the passages, the webs nearly always sloped at about 45° upwards and inwards towards the stack. The older spiders built their webs nearest to the front of the gap between dumps, as seen from the passage, while young ones built their smaller webs deeper in, where the faces of the dumps were closer together. In the centre of each web was a circular or oval "sheeted hub" of dense, white, semi-opaque webbing, densest towards its margin. The spider clung to the underside of this, with the tips of its long first pair of legs alone projecting beyond it.

The prey of *U. geniculatus* appeared to consist mainly of clothes moths, and indeed no other potential prey was present in the numbers necessary to maintain the enormous spider population. Although prey was very rarely seen suspended in the webs or being consumed by the spiders, the remains of moths were to be found accumulated under the webs—on the floor, on projecting shoulders of dumps, and in old webs. The remains of each moth formed a characteristic small, silk-covered sphere about 2 mm in diameter, with a pit in it. The spider thoroughly macerates the tissues, for appendages etc. are not evident on rapid examination with a lens. The pit is presumably produced by the spider's mouthparts.

*Identification by Mr. G. E. J. Nixon, Commonwealth Institute of Entomology, London. Specimens deposited in the Division of Entomology Museum, C.S.I.R.O., Canberra.

†Identification by Mr. A. Musgrave, Australian Museum, Sydney. Specimens deposited in the Australian Museum.

The eggs of *U. geniculatus* were contained in a strongly flattened, pale mauve, silken sac about 1 cm in diameter, which was suspended in the web, usually 2–8 cm above its centre, by a variable number (frequently seven) of marginal points. The eggs usually adhere together as a small opaque sphere within the sac, but may also be disposed loosely within it. After hatching, the spiderlings apparently remain within the sac for a time.

Counts were made in March 1944 of the spiders in the gaps between the vertical faces of adjacent dumps from the floor to the top of the second tier (i.e. a height of about 5 ft), those that were too small to bridge the gap with their webs being ignored. The figures for 10 such gaps in different parts of the store ranged from 19 to 40, with a mean of 29·8, or *c.* 6 per foot.

Ants were present in the store, especially near the walls, but were not prominent. Small lizards were also often seen. Miscellaneous insects were occasionally taken on the tanglefoot traps in very small numbers (various moths, a few termites, and a few small Coleoptera, Hymenoptera, and Diptera). One or two cats were allowed to roam the store to discourage rats, of which no evidence was seen.

III. Life Cycle: Distribution of the Different Stages

External evidence of infestation of a dump by moth is afforded by the presence of frass, silk, or cocoons on the wool protruding through breaks in the pack. Cocoons may also be scattered over the general surface, especially in the furrows produced by the folding of the pack. When the Brisbane stores were first examined in December 1941, the infestation appeared to be mainly confined to the protruding wool, although it had frequently spread some inches laterally between the pack and the wool, particularly on faces II and III where the pack is thrown into folds (see Section II(*b*)). Small infestations were also found under the pack at points apparently unconnected with the breaks. In April 1942, dumps were examined in which the infestation was much more general, frass and silk being densely and more or less uniformly distributed over the surface of the wool beneath the pack.

A marked difference was noted in the degree of infestation of the different faces of the dumps. Face III was much the most heavily infested, one bale showing evidence in April of many hundreds of larvae. Face II was next, with I somewhat lower. This order of infestation corresponds with the order of degree of compression suggested in Section II(*b*). Faces II and III are probably rendered more favourable by the presence of spaces between the pack and the wool where it has been folded under compression, and face III may have an advantage on account of its horizontal orientation in the stack, which results in a closer approximation of this face to the corresponding faces of the dumps above and below.

In the course of thorough examinations of many dumps, both in C store and elsewhere, no evidence has been found of larvae occurring at a depth greater than 2 in., except for one larva on what was originally the wool surface at the head of one of the constituent bales. It is probable that the moths first entered the stores as a very low-level infestation of the incoming wool, in which case various life-cycle stages must have been entombed in the dumps at dumping time. The evidence

indicates that such insects were unable to survive, or at least to reproduce, and that subsequently the infestation was confined to the surface 2 in. of the wool.

Both on protruding wool and on the surface of the wool beneath the pack, the infestation was densest around pieces of faeces, sweaty tips, or burrs. The faeces and sweaty tips were eaten by the larvae and presumably parts of the burrs also. These observations are in accord with the general experience that the better-quality, cleaner, greasy wool was less heavily infested than the contaminated low-grade types, and scoured wool less heavily than greasy. It agrees also with much published information (cf. Titschack 1922; Griswold 1944; Cheema 1956) showing that pure wool is a most unsatisfactory food for both *Tineola* and *Tinea*, but that it may be made nutritionally adequate by contaminants of both animal and vegetable origin.

The infestation in the wool underneath the pack consisted almost entirely of *Tineola*, larvae of *Tinea* being practically confined to the loose protruding wool, where *Tineola* also occurred (cf. Griswold 1944). No doubt the case-bearing habit of the *Tinea* larva prevents it from burrowing into compressed materials. In collections of loose wool, such as have been examined at the Central Technical College, Brisbane, and at the Veterinary School, University of Queensland, *Tineola* was absent or rare, while *Tinea* was prominent. Moreover, we are informed by Mr. F. A. Perkins, University of Queensland, that a culture on loose wool started by him with adult moths collected at random in C store proved after some months to contain only *Tinea* larvae. Since *Tineola* was very abundant in the store, this observation suggests that its larvae may be unable to compete with *Tinea* when deprived of the protection of a relatively dense medium in which to tunnel.

Under the wool pack, the tunnels of *Tineola* commonly showed at first a certain wandering at the surface of the wool and then entered the wool mass perpendicularly to a depth of 1–2 in. Almost all the larvae collected from opened dumps were found at the inner end of these tunnels. Frass is pushed towards the outer part of the tunnel and accumulates, along with silken webbing, around its mouth. On the extremely taut face I there is no room under the pack for superficial wandering, or for webbing or frass, and the only evidence of the presence of a larva was usually a neatly excavated hole leading directly into the wool mass.

Pupation of *Tineola* usually occurred either at the surface of the wool, or on the outer surface of the pack, which was reached by the larva biting its way through the pack fibres, thereby leaving a characteristic small round hole. Cocoons on the outside of the pack were largely constructed of pack fibres. The choice of site for pupation was probably determined by the amount of space between the pack and the wool. Where this was adequate, for example among folds, pupation and the subsequent emergence of adult moths could take place under the pack; quite a number of apparently healthy, normal moths have in fact been found on a single face in this situation. There seems no reason why they should not be able to reproduce there.

The great majority of the adult moths undoubtedly emerged outside the pack. They could be seen at any time sitting or running on the dumps, especially when the darker recesses of the stacks were illuminated with an electric torch, and

making short flights from dump to dump. At about dusk, flight activity greatly increased (Section V(*a*)) and large numbers of the males of both species were in free flight in the passages as well as within the stacks. The observations of earlier authors on the two species concerned (cf. Titschack 1922; Cheema 1956) were confirmed in that females only rarely participated in these flights (Section V(*b*)). The few that did sometimes contained mature eggs and sometimes not. It seems clear that, in general, the movements of the females were confined to crawling over the surfaces of the dumps and that they did not move any great distance from their point of emergence. If this is so, the general infestation in C store and the other infested stores must have developed from a rather uniformly distributed, even if sparse, pre-existing infestation of the wool, rather than from a few widely scattered foci.

It is probable that the females for the most part laid their eggs on wool protruding through major breaks in the pack or accessible through wool-hook holes. On the other hand, in view of the observed occurrence of infestations of *Tineola* at points remote from obvious openings, there seems little doubt that its larvae can gain access to the wool even under an intact pack. A female moth has indeed been observed sitting on a dump with its abdomen flexed towards the pack, as though ovipositing on or through it.

Already in December 1941 the great majority of the dumps in C store from the 1939–40 and 1940–41 clips were infested in varying degree, and by 1943 probably no dump was free from infestation. The infestation extended right through the stacks, being certainly no less in their centres than along the passages. Although, as has been mentioned, some dumps contained evidence of many hundreds of well-grown larvae, an average figure for the store by 1943 would probably have been about 50 per dump. It is probable that the amount of wool consumed, even in the most severely infested dump, never exceeded 0·1 per cent. of the total.

It was not possible to determine with certainty the number of generations passed through per annum. Evidence bearing upon this point will be discussed in Sections VII and VIII.

IV. Technique of Sampling Adults

Systematic sampling of the larvae as a means of studying spatial and temporal differences in the degree of infestation was impracticable, for there was no way of forming any estimate of the number of larvae in the interior of a stack without dismantling it—a procedure that would have been neither desirable nor feasible. Attention was therefore concentrated upon sampling of adults. Preliminary experiments showed that the moths could be caught in numbers on surfaces coated with tanglefoot. Tanglefoot traps, which have theoretical advantages over traps employing any type of lure and are well adapted for use in large numbers and within confined spaces, were therefore made the basis of the sampling technique.

(*a*) *Sampling in an Alley*

For the purpose of establishing a relation between the number of moths trapped and the levels of relevant environmental factors, over a short period within which it could be assumed that the population level had remained constant, a

simple method of trapping large numbers of moths was required. A cubical trap with a side of 2 ft was constructed by covering each face of a stout wooden frame with a sheet of 24-gauge galvanized iron and coating the entire surface of the cube, as evenly as possible, with tanglefoot.* The trap was suspended in alley 4R by cords (Fig. 1; Plate 3, Fig. 1), so that it hung half way between the floor and the top of the stacks, *c.* 1 ft from stack 5R and 3 ft from 4R. It was *c.* 25 ft from a window high up in the west wall and *c.* 95 ft from a corresponding window at the other end of the alley. The various faces were thus differentiated with respect to light intensity, distance from the stack face, and vertical or horizontal orientation. The thermohygrograph referred to in Section II(*c*)(i) was fixed to a board bridging the alley from stack to stack with its sensitive elements opposite the centre of the trap at a distance of a few feet from it.

The cubical trap was installed on April 9, 1942, and was in use continuously until May 24, 1943. After a gap of some 5 months its tanglefoot coating was renewed and trapping resumed between October 27, 1943, and December 13, 1943. Normally the catch was counted and removed daily during the forenoon. At that time of day exceedingly few moths are caught (Section V(*a*)), so that the variation of up to 5 hr in the exact time of counting could have introduced only a very small error. As between successive days, this variation rarely exceeded 1–2 hr. The moths were removed from the tanglefoot for subsequent separation of the species and sexes,† the surface of the tanglefoot being smoothed over where necessary immediately afterwards.

During the first few months of trapping the performance characteristics of the trap were analysed with respect to the distribution of the catch over each face and as between faces. Various significant trends were found, indicating that the catch of a tanglefoot-covered surface will vary according to its orientation. However, the interaction variance of species with face (as of face with week) was not significant over a trial period of 6 weeks during which 2503 moths were caught. Thus the ratio in which the two species are caught may be expected to be unaffected, within wide limits, by differences in the design or placement of a tanglefoot trap.

The influence of accumulation of moths already caught upon the catching capacity of the trap was also examined by allowing moths to accumulate on one half of each face while removing them daily from the other. It was concluded that, where the daily catch is not less than 0·025 moth per sq. in., a reduction (of *c.* 20 per cent.) in daily catch may be expected as between regimes of daily clearance and of clearance at greater than daily intervals, but not, at least up to a total accumulation of 0·13 moth per sq. in., as between regimes of clearance at different intervals of more than a day.

(*b*) *Sampling within a Stack*

For sampling within a stack, small tanglefoot traps were used which could be lowered down the vertical "funnels" of the stack (see Section II(*b*)) from above.

*The tanglefoot used was sold under the trade name of "Ostico".

†The genitalia were used for determining sex.

Different designs of such traps were tested in the course of trapping in four adjacent funnels in stack 5R, a moderately infested stack of 1940–41 wool standing five dumps high (Fig. 1). The traps comprised four sizes of square galvanized iron

TABLE 3

THREE-HOURLY CATCHES OF TINEOLA AND TINEA ON THE CUBICAL TRAP BETWEEN 6 P.M. ON APRIL 10, 1942, AND 6 P.M. ON APRIL 13, 1942

Sunset 5.36 p.m.

Date	Species	Three Hours Ending:							
		3 a.m.	6 a.m.	9 a.m.	12 Noon	3 p.m.	6 p.m.	9 p.m.	12 Midnight
10.iv.42	*Tineola*							29	1
	Tinea							4	0
	Total moths							33	1
11.iv.42	*Tineola*	2	0	1	0	5	11	24	5
	Tinea	0	0	0	0	0	1	6	1
	Total moths	2	0	1	0	5	12	30	6
12.iv.42	*Tineola*	3	1	0	1	1	6	21	1
	Tinea	1	0	0	0	0	0	9	0
	Total moths	4	1	0	1	1	6	30	1
13.iv.42	*Tineola*	2	1	0	0	4	1		
	Tinea	0	0	0	0	0	0		
	Total moths	2	1	0	0	4	1		
Totals	*Tineola*	7	2	1	1	10	18	74	7
	Tinea	1	0	0	0	0	1	19	1
	Total moths	8	2	1	1	10	19	93	8

plates 9, 16, 36, and 64 sq. in. in area, and three types of galvanized iron cylinders respectively 6 in. long by 2 in. dia., 4 by 3 in. dia. (these two having a surface area of 37·7 sq. in.), and 3 by 2 in. dia. (area 18·8 sq. in.). The external surface of the cylindrical traps and one side of the square traps was coated with tanglefoot and these faces were protected from contact with the dumps by wires (Plate 3, Fig. 2).

The traps were suspended at four different levels in each of the funnels and the catch counted and removed every few days. After each exposure the arrangement of the traps was altered until each type of trap had been used once in each position. The catches obtained were subjected to an analysis of variance. Aggregation was found to be of negligible importance. Within the group of plate traps, and again within the cylindrical traps, the catch did not depart significantly from proportionality to trap area, but the catch per sq. in. per day differed significantly between the two groups, the mean for the cylinders being about 50 per cent. above that for the plates. For this and other reasons, the cylindrical type of trap was accepted as more efficient than the plate type, and further sampling within stacks was conducted with 6 by 2 in. cylinders.

TABLE 4

HALF-HOURLY CATCHES OF MOTHS ON THE CUBICAL TRAP BETWEEN 4.30 AND 7.30 P.M. OVER THE PERIOD MAY 11–15, 1942, WITH THE 24-HR CATCHES COMMENCING 10 A.M.

Sunset 5.9 p.m.

Date	Half-hour Ending:						24 Hours Commencing 10 a.m.
			5.50 p.m.	6.20 p.m.	6.50 p.m.	7.20 p.m.	
11.v.42			12	3	5	0	46
	5.00 p.m.	5.30 p.m.	6.00 p.m.	6.30 p.m.	7.00 p.m.	7.30 p.m.	
12.v.42	1	4	5	7	1	—	29
13.v.42	3	5	8	9	6	0	59
14.v.42	3	3	1	2	1	—	29
15.v.42	0	5	3	2	1	—	21

V. BEHAVIOUR OF THE ADULT MOTHS

General observations on the behaviour of adults have already been reported in Section III. In the present section data obtained by trapping will be considered. These relate more particularly to the distribution of flight activity within the 24 hours, the relation between flight activity and temperature, and the distribution of flying moths within the stacks and as between stacks and passages. The sampling techniques described in the preceding section were employed.

(*a*) *Flight Period*

Between 6 p.m. on April 10, 1942, and 6 p.m. on April 13, 1942, the moths caught on the cubical trap were counted and removed every 3 hr. In each 24-hr period (Table 3) the catch of both *Tineola* and *Tinea* was found to be markedly

concentrated into the three hours ending 9 p.m., i.e. there was a marked flight period around dusk, with its peak at about 7.30 p.m. In *Tineola*, although some 62 per cent. of the total catch was made between 6 and 9 p.m., quite appreciable catches were registered in other of the 3-hr periods, and it was only between 6 a.m. and noon that the figures fell to a very low level. In *Tinea*, on the other hand, the concentration was more intense, some 86 per cent. of the catch being made between 6 and 9 p.m. This difference between the species is significant.

In order to define more precisely the time of maximal flight activity, half-hourly observations were made during the late afternoon and evening over the period May 11–15, 1942, and again on November 23 and 24, 1942. To reduce disturbance to a minimum during this critical period, the trap was approached very cautiously, the catch was counted as rapidly as possible, and the moths were not removed, the number caught during each period being obtained by difference. It will be seen from Tables 4 and 5 that the time of the flight peak varied consider-

TABLE 5

HALF-HOURLY CATCHES OF MOTHS ON THE CUBICAL TRAP BETWEEN 5.15 AND 8.15 P.M. ON NOVEMBER 23 AND 24, 1942, WITH THE 24-HR CATCHES COMMENCING 8 A.M.

Sunset 6.22 p.m.

Date	Half-hour Ending:						24 Hours Commencing 8 a.m.
	5.45 p.m.	6.15 p.m.	6.45 p.m.	7.15 p.m.	7.45 p.m.	8.15 p.m.	
23.xi.42	3	2	10	18	29	6	115
24.xi.42	—	20	25	14	6	3	182

ably, not only as between the May and November observations, but also within each of these groups. On both days in November the peak may be considered to have fallen within the 6–9 p.m. period (even on the basis of three-hourly totals) as it did in the April observations, but it was a full hour earlier on the 24th than on the 23rd. In May, although the peak fell within the 6–9 p.m. period on the 12th and 13th, it fell in the 3–6 p.m. period on the remaining days, and on all five days the 3–6 p.m. total would have exceeded the 6–9 p.m.; the time of the peak again ranged over at least an hour as between different days. A rough estimate may be made of the percentage of the total 24-hr catch that fell in a 3-hr period including the peak half hour. On most days this would seem to be somewhat lower than the 65 per cent. given by Table 3 for the two species together. The data of Tables 3–5 suggest that the curve of catch with time during the flight period is asymmetric, dropping away more steeply after the peak than before.

The variability in the time of maximal flight activity is somewhat reduced if for each day we express this time as the difference between sunset and the mid-point of the period of maximal catch. Although the data are far from adequate, they do suggest (Fig. 5) that the variability may be due to the interaction of a light

factor with temperature. It seems that the higher the temperature, the later may be the peak, or, conversely, that low temperature enforces an early peak. On this interpretation the line in Figure 5 would represent the longest interval after sunset at which the peak can fall at indicated temperatures.

Evidence to be presented in the next section will show that the 24-hr catch increases with the temperature level during the flight period up to about 80°F. During all three sets of observations the temperature had already fallen below this level by dusk. Thus temperatures were becoming progressively *less* favourable for flight during the flight period. The fact that even in May, when the most favourable temperatures occurred in the middle of the day, the flight peak did not occur until sunset or later points to the significance of a light factor: either a

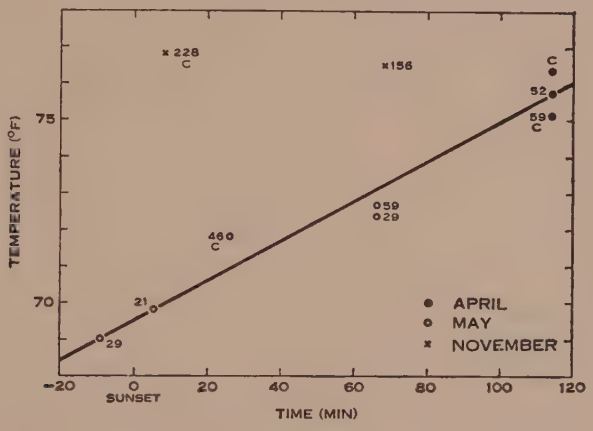

Fig. 5.—Relation between the time of maximal flight activity of the moths in minutes after sunset, the temperature at that time, and the catch in the corresponding 24-hr period (figures associated with each point) for individual days in April, May, and November, 1942. The regression line takes account of the April–May observations only. *C*, cloudy.

particular low light intensity or, more probably (cf. Waloff and Richards 1946; Common 1954), a rapidly falling low intensity. Thus light conditions may be considered to be becoming progressively *more* favourable over the period following sunset. The situation suggested by Figure 5 is therefore what one would expect: when the temperature remains high enough during the evening the peak activity tends to be deferred until late, when the light conditions are "optimal"; when the temperature is lower, the time of the peak depends upon the resultant effect of the steadily deteriorating temperature and the improving light conditions, and in general is earlier the lower the temperature.

The occurrence of a marked flight period in the two clothes moths means that in sampling work it is preferable to employ exposure periods of a whole multiple of 24 hr and to avoid making counts or inspections during the afternoon or evening. The most satisfactory period for inspections is the forenoon, when considerable variation in the precise time of counting can be tolerated without introducing an appreciable error.

(*b*) *Activity and Temperature*

Examination of the records from the cubical trap shows that, in addition to long-term trends in the catches of both species, which may be interpreted as resulting from changes in the number of adult moths in the store, there are marked day-to-day fluctuations that can only be due to changes in flight behaviour, including such aspects as proportion of moths in flight, average flight distance, and average flight speed. Such behaviour differences are most likely to be due to microclimatic differences between successive days, particularly within the flight period; the factor most likely to be effective is temperature.* An analysis was therefore made of the relation between catch and the mean temperature during the flight period, which has been taken as the mean of the temperatures recorded by the thermograph at $\frac{1}{2}$ hr and $2\frac{1}{2}$ hr after sunset. Owing to the relatively standard character of the temperature regime, a somewhat different delimitation of the flight period would make little difference to the relative temperatures of different days. The figures for males only were used and the two species treated separately by a method similar to that of Williams (1940).

Data for consecutive days were grouped, subject to certain restrictions, to reduce the sampling error. The restrictions were (1) that the number of days should preferably be not more than 3 or 4, although on occasion as many as 7 were grouped, and (2) that the range in temperature within a group should be small, generally less than 2°F. The mean catch and mean temperature were calculated for each group and the logarithms of the mean catches taken. The differences between consecutive groups in log mean catch and in mean temperature were then computed (the order in differencing being such as to make the temperature difference always positive), also the mean temperature for each pair of consecutive groups (mean of the unweighted group means). The data on differences were classified, according to the mean temperature, in 2°F ranges. Within each range the separate differences were then combined, with weights which were, for each difference, the reciprocal of the sum of the reciprocals of the number of days in the two groups concerned. The calculation thus gave for each range a total of weighted differences of log catches and a total of weighted temperature increments. The ratio of these gave a measure of the rate of increase in log catch with temperature at the mean temperature of each range.

At this point an analysis of variance of the ratios found for *Tineola* and *Tinea* was carried out and showed that the responses of the two species were not significantly different. The whole calculation was therefore repeated, using the catches for the two species combined. A quadratic regression relation of the resulting ratios against mean temperature was fitted by least squares. This gave

$$\mathrm{d}(\log N)/\mathrm{d}T = 0{\cdot}067687 - 0{\cdot}003484T - 0{\cdot}000756T^2,$$

from which

$$\log N = 0{\cdot}067687T - 0{\cdot}001742T^2 - 0{\cdot}000252T^3 + K.$$

*Griswold (1944) has already noted that *Tineola bisselliella* adults are most active at high temperatures.

Temperature was measured in 2°F units from 65°F as zero. Taking N to be 1 at $T = 0$ and substituting for other values of T, we can arrive at a relation between an "activity index" (relative catch) and temperature, based on short periods within which it may be assumed that the number of adult moths in the store had remained relatively constant. This relation is represented in Figure 6.*

It may be seen that rise of temperature increases the catch up to about 80°F, after which it reduces it. A rise from 65 to 80°F approximately doubles the catch.

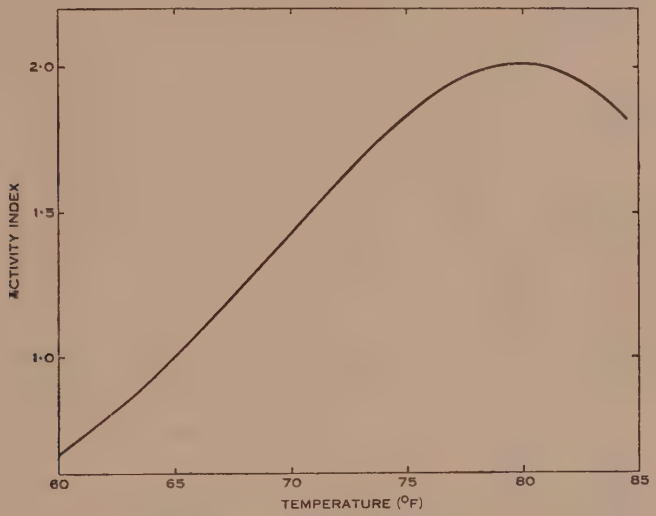

Fig. 6.—Relation between flight-period temperature and flight activity of the moths, referred to 65°F as standard. For details see text.

If we use the regression equation to correct the daily catches of males of the two species separately to a standard temperature of 65°F, it is found that much clearly non-random variability within short periods is retained by the catch data for both species. Thus on October 25, 1942, the corrected catch of *Tineola* males was 449 and on the following day 103. On at least six days where the corrected catch for *Tineola* exceeded 30 (on several it was well over 100) it was equal to, or greater than, the catch of the preceding and following days combined. On each of these days the catch of *Tinea* showed the same relation. These deviations are clearly due to some factor or factors influencing flight behaviour in the same way in the two species, but we have been unable to identify such factors. It might be supposed that on the days concerned the flight period had occurred at an unusual time coinciding with temperatures higher than those prevailing during the "standard" flight period. However, to cause a doubling of the catch in relation

*The method used is not altogether satisfactory. The need to make comparisons over short periods implies small differences in both log catch and temperature. The former has a relatively large sampling error and probably the latter also, understanding the error in this case to be the departure of the recorded temperature from a weighted mean over the store and over the actual flight period for the day concerned.

to that of the preceding or following day, the relevant temperature would need to be some 15°F higher, a difference that would not be realized even if the flight period were to shift to the time of daily maximum temperature. The characteristics of the days concerned have been examined with respect to maximum and minimum temperature, temperature during the 24-hr period preceding that in which the relevant flight period fell, steepness of the temperature fall during the flight period, relative humidity, cloud amount, phase of moon, dawn conditions, presence or absence of females on the trap, and disturbance due to the periodical removal of wool from the store. No correlation with any of these factors was apparent. A possible explanation is that there may be a tendency to flight aggregation, or incipient swarming. In that case it would be a matter of chance whether a "swarm" encountered the trap. We have formed the impression that noticeable local differences do exist in the density of flying moths. Herrick (1933) has reported migration in *Tineola*, but it is not clear from his paper whether this was accompanied by any manifestations of gregariousness.

The very low flight activity of the females of both species has already been mentioned. The percentage of females in the catch on the cubical trap was calculated for the species separately by months or suitable groups of months over the whole period of operation of the trap. On the average, the figure for *Tinea* was about 1·5 times that for *Tineola*, but the difference was not significant. Combining the data for the two species, the female percentage was found to remain relatively constant over the period April to December 1942; 10,830 males were caught and 30 females, giving a percentage of 0·28.

According to Titschack (1936) and Griswold (1944), adult *Tineola bisselliella* emerging from cultures in which the larvae have been able to obtain an adequate quantity of food show a slight preponderance of females. Variation in the quality of the food does not affect the sex ratio on emergence (cf. Griswold 1931, 1944), but an inadequate quantity of food results in an increase in the proportion of males, which may reach a level of 2 : 1, or even higher under very severe food limitation (Titschack 1936). In the store, the average degree of crowding of the larvae was probably never such as to give rise to a sex ratio much above 1 at emergence. Titschack (1926*a*), working at 30°C, showed that *Tineola* males had an average adult life about 1·7 times as long as fertilized females. Similar results were obtained by Griswold (1944) at lower temperatures. Thus in an established breeding population the ratio of males to females at any time should be about 1·7 times the ratio at emergence; for the store we may assume a value of about 2·0. On this basis a female percentage of 0·28 in the catch of *Tineola* corresponds to a "catchability", or relative flight activity, of females amounting to about 0·6 per cent. that of males.

If the results of Cheema (1956) on sex ratio and adult life span in *Tinea pellionella* are truly comparable with those just cited for *Tineola* (as they would seem to be), then the two species are rather different in these respects. Cheema reports a ratio of 1 male to 2·6 females on emergence and a female life span 1·5 times that of the male. This gives a ratio of about 1 male to 4 females in an established population and a relative flight activity of females 0·07 per cent. that of males, i.e. about one-tenth that in *Tineola* (relative, in each case, to the male). It must

be regarded as a matter of chance that the interaction of the sex ratio and the relative activity of the sexes in *Tinea* leads to a value for the percentage of females in the catch very close to that obtained for *Tineola*.

After December 1942 the female percentage rose steadily up to November 1943 (Table 9). This is believed to have been due primarily to a disturbance of the sex ratio, resulting from an increasing differential mortality of adult males (Section VII). If this interpretation is correct, it is probable that fertilization of the females would tend to be delayed, and such females would live somewhat longer. However, since completely unfertilized females were found by Titschack (1926*a*) to live only 1·5 times as long as fertilized, the contribution this effect could make to the rise in the female percentage would be slight. If a low male/female ratio were to result in increased female activity or reduced male activity, then this could also be a contributant, but we have no evidence of such an effect.

During the trapping in stack 5R (Section IV(*b*)) 765 males of both species and three females were caught, giving a female percentage of 0·39, a value quite comparable with that for the passage during the same period. This indicates that short-range flight within the stacks is almost as rare among females as flight into the passages and suggests that, once the resistance to taking flight has been overcome, the female may fly almost as strongly as the male. Females have been taken on various faces of the cube, not only on that nearest to the nearer stack, and can probably fly from stack to stack.

The data on females are not adequate for a study of the effect of temperature on flight activity; however, females of both species were caught over almost the whole range of temperatures experienced. As we have seen, the female percentage did not vary with the season, but remained relatively constant during 1942 and rose steadily during 1943. There is thus no reason to suppose that temperature has an effect on the female different from its effect on the male.

(*c*) *Distribution*

The trapping carried out in stack 5R between February and April, 1942 (see Section IV(*b*)) gives information on the variation in catch within a typical stack. During the week April 12–19, 1942, it overlapped with the first week of operation of the cubical trap, situated immediately adjacent to the row of funnels in which the stack traps were exposed. Thus for this period a comparison can also be made between the catch in the stack and in the alley.

The total catch (both species together) of the cylindrical traps in the stack during the week of overlap was 49 moths, or 0·019 moth/sq. in./day. The corresponding figure for the four vertical faces of the cubical trap was 275 moths, or 0·020 moth/sq. in./day. Assuming that the catching capacity per unit area of cylinder would be closely similar to that of the vertical faces of the cubical trap taken together, we may infer that the average density of flying moths was the same in the air spaces of the stack as in the adjacent alley. Although we have no accurate information as to the relative volumes of these two spaces, it would seem that a substantial proportion of the moths that took to flight must have entered the passages during each flight period. The percentage of *Tinea* in the stack catch

was 27·1 and in the alley 26·4. We may conclude that *Tinea* entered the passages to the same extent as *Tineola*.

Table 6 shows the catches of males of both species, totalled for funnels and levels, over two exposure periods in the stack. Although the catch in funnel 1 (nearest the alley: see Fig. 1) was consistently higher than the others, the difference does not attain significance. On the other hand, in *Tineola* there was a marked gradient in the size of the catch from the lowest tier (level *a*) to the top of the stack (level *d*), which is significant at $P<0{\cdot}001$ for both exposures. There is a suggestion of a similar, but less steep, gradient in *Tinea*, but this is not significant.

In the autumn of 1943, trapping was undertaken in the experimental stack already referred to (Section II(*c*)(ii)), using initially 20 traps distributed throughout

TABLE 6

CATCHES OF TINEOLA AND TINEA MALES, TOTALLED FOR FUNNELS AND LEVELS, IN STACK 5R DURING FEBRUARY–APRIL, 1942

Species	Exposure	Funnels				Levels			
		1	2	3	4	*a*	*b*	*c*	*d*
Tineola	A	53	32	33	26	10	21	42	71
	B	98	71	60	69	33	55	86	124
	A+B	151	103	93	95	43	76	128	195
Tinea	A	8	6	7	2	3	6	7	7
	B	17	12	14	16	7	17	20	15
	A+B	25	18	21	18	10	23	27	22
Species combined	A+B	176	121	114	113	53	99	155	217

the stack, with four at each of the five levels between tiers of dumps, and later 100 traps, with 20 per level. The catch was allowed to accumulate for periods of 3 or 4 days and then counted and removed. The results may conveniently be considered in three separate periods. Table 7 shows a marked gradient from bottom to top in the catch during the first period and a less marked one during the second; both are significant at $P<0{\cdot}001$. The third period shows no significant variation with level.

The experimental stack had been erected only shortly before the commencement of trapping and consisted of a more or less random arrangement of infested dumps. There was therefore no reason to expect a significant difference in the degree of infestation of the wool in the different tiers. Thus the higher catch in the upper part of the stack must have been due either to greater activity of the moths at these levels, or to a tendency for moths to concentrate there. The temperature data presented in Figure 2(*a*) and Tables 1 and 2 suggest that the difference in temperature between the tops of the first and fifth tiers (i.e. between levels *a* and *e*) during the

flight period is unlikely to have reached 10°F on any one day, and from Figure 6 we see that this would correspond to an increase of activity in the ratio of about 1·4. This is approximately the ratio of the catches at levels *a* and *e* during the second period in Table 7, but is much exceeded by the corresponding ratio for the first period (about 2·5). The latter, in fact, could not possibly be accounted for by the effect of temperature on activity. Thus it seems that there was some concentration of moths in the upper part of the stack during the first period and probably to a less extent during the second, but that by the third period this tendency had disappeared.

Returning to the data for stack 5R (Table 6), we see that the ratio between the catches for the two species combined at levels *a* and *d* is about 4, i.e. considerably greater than between levels *a* and *e* at any period in the experimental stack. It seems likely that the accumulation of moths in the upper levels postulated for the experimental stack is connected with the temperature gradient within the stack

TABLE 7

MOTH CATCHES AT DIFFERENT LEVELS IN AN EXPERIMENTAL STACK RECENTLY ERECTED FROM INFESTED DUMPS

Level *a* the lowest, *e* the highest

Period	Level				
	a	*b*	*c*	*d*	*e*
21.ii .43–8.iii.43	57	47	83	105	142
19.iii.43–1.iv.43	81	71	91	117	110
1.iv.43–2.v .43	110	89	83	108	100

and that its progressive elimination during the second and third periods is to be ascribed to a reduction in that gradient as a result of the reduction in insolation and external temperature as the autumn advanced. A similar reduction in the steepness of the catch gradient (for *Tineola*) is to be observed between the first exposure (February) and the second (March–April) in stack 5R. If this explanation is correct, the steeper temperature gradient in stack 5R than in the experimental stack (Section II(*c*)(ii)) may have caused the steeper catch gradient by increasing the accumulation. It is probable that a persistent density gradient of the adult moths (if, as is likely, it extended to the females also) would tend to produce a higher level of infestation in the wool of the upper tiers. However, the great mobility of the males, evidenced by all the trapping observations, indicates that such an infestation gradient could not contribute significantly to the catch gradient.

Even if we accept as real the non-significant gradient of the *Tinea* catch with level, the ratio of level *d* to level *a*, for the two exposures combined, is only 2·2, as against 4·5 for *Tineola*. Although this difference in ratio has not been tested for

significance, it suggests that *Tinea* did not concentrate in the upper levels to the same extent as *Tineola*.

From the above data we may draw certain conclusions as to the distribution of the adult moths in the store. The males are so mobile that within each species their density during the flight period was probably much the same, at any given horizontal level, throughout the stacks and passages. However, during the warmer months there was in *Tineola*, and perhaps to a less extent in *Tinea*, an upward gradient in the density of males within the stacks and presumably also in the passages, this being probably connected with the corresponding temperature gradient. After the flight period the moths withdrew into the stacks; we have no

TABLE 8

THREE-HOURLY CATCHES OF APANTELES CARPATUS ON THE CUBICAL TRAP BETWEEN 6 P.M. ON APRIL 10, 1942, AND 6 P.M. ON APRIL 13, 1942

Date	Three Hours Ending:							
	3 a.m.	6 a.m.	9 a.m.	12 Noon	3 p.m.	6 p.m.	9 p.m.	12 Midnight
10.iv.42							1	0
11.iv.42	0	0	0	0	2	0	0	0
12.iv.42	0	0	0	0	6	2	2	0
13.iv.42	0	0	1	0	10	3		
Total	0	0	1	0	18	5	3	0

information on their distribution there. To the extent that females participated in the flights, they probably showed the same distribution as the males. However, exceedingly few of them did, and the distribution of the females was probably in general much less uniform than that of the males, varying from stack to stack, as well as within stacks, according to the density of the immature stages. The latter would undoubtedly be largely influenced by the degree of exposure and contamination of the wool and by temperature and humidity, among other factors.

VI. BEHAVIOUR OF ADULT APANTELES CARPATUS

Throughout the period of operation of the cubical trap, records were kept of the catches of the parasite *Apanteles carpatus* (see Section II(*d*)). These allow conclusions to be drawn on flight behaviour and fluctuations in abundance of the adults.

(*a*) *Flight Period*

Table 8 shows the catches obtained in successive 3-hr periods between 6 p.m. on April 10, 1942, and 6 p.m. on April 13, 1942 (cf. Table 3). In each 24-hr period

the bulk of the catch was made between noon and 3 p.m. The figures suggest that the actual peak must lie nearer to 3 p.m. than to noon, i.e. very close to the time of daily maximum temperature (Fig. 2(*a*)). There is thus no reason to postulate a flight period triggered by light intensity; rather does it seem that the time of maximal activity is determined by a simple relation to temperature.

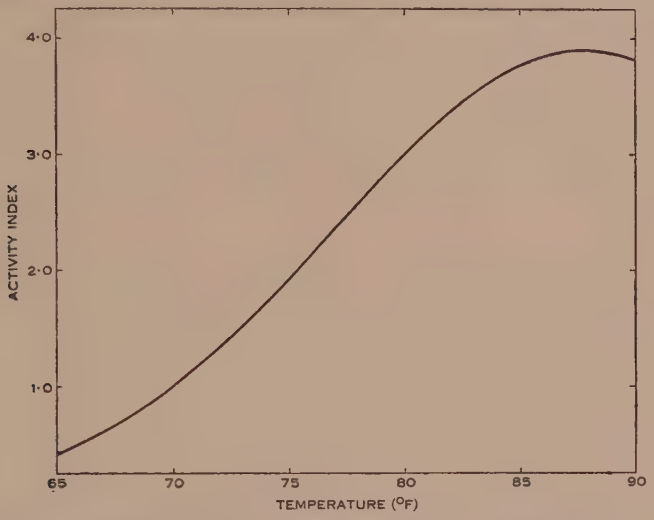

Fig. 7.—Relation between daily maximum temperature and flight activity of *Apanteles carpatus*, referred to 70°F as standard. For details see text.

(*b*) *Activity and Temperature*

The relation between daily catch and temperature was analysed for *Apanteles* by a method similar to that used for the moths (Section V(*b*)), taking the daily maximum reading of the thermograph as the measure of temperature. A series of ratios, representing rates of increase in log catch with temperature at 2°F intervals was obtained in the same way. The quadratic regression of these ratios on temperature, obtained by least squares, shows the rate as rising over the last three intervals, corresponding to the upper part of the temperature range. This rise probably has no biological meaning; it is largely due to the high ratio for the highest temperature interval, which, however, is based on differences in only two pairs of data groups (see Section V(*b*)). A linear regression was therefore fitted in place of the quadratic. This gave

$$\mathrm{d}(\log N)/\mathrm{d}T = 0{\cdot}125760 - 0{\cdot}015026T,$$

from which

$$\log N = 0{\cdot}125760T - 0{\cdot}007513T^2 + K.$$

A temperature of 70°F was taken as zero for the purpose of arriving at a relation between an activity index and temperature, since for individual days a maximum of this value corresponds rather well with the figure of 65°F during the flight period of the moths (this value having been adopted as the standard for the activity index of the moths). Taking N to be 1 at $T = 0$ and substituting for other values of T, we arrive at the relation represented in Figure 7.

Fig. 8.—Fluctuations in the abundance of adults of *Tineola bisselliella*, *Tinea pellionella*, and *Apanteles carpatus* in C store between February 1942 and December 1943. Data based on the daily catches of the cubical trap, corrected for the effect of temperature on activity, averaged over usually fortnightly periods, and expressed in the transformation log $(n+1)$. Also, number of females per 1000 moths in the catch of the two species combined, and abundance of *Tineola* calculated on the assumption of no spider predation, both expressed in the same transformation. Above, mean daily maximum temperature, with (numerals) the number of days in each period when temperatures lethal to the moths might be expected in the upper tiers of the stacks. Estimates from incomplete data shown in thinner line. For details see text.

The form of this curve is similar to that of Figure 6. The peak lies at about 88°F. On a day with a maximum temperature of this value the mean temperature during the flight period of the moths would be close to the figure of 80°F given by Figure 6 as the temperature of maximal moth activity. Thus in general the moths and their parasite may be expected to reach high levels of activity on the same days. However, a 15°F rise of temperature above the standard increases the parasite catch by a factor of about 3·8, as against 2·0 for the moths.

The parasite catch was not large enough to allow us to determine whether there was significant residual variance within short periods after the daily catches had been corrected to the standard temperature. Comparison with the corrected moth data shows, as would be expected, no correlation between the two groups in their short-period variation.

VII. Population Changes of Moths and Apanteles

The daily catches of each species of moth and of *Apanteles carpatus* on the cubical trap, when corrected for the effect of temperature on activity (to 65°F as standard in the case of the moths and 70°F in the case of the parasite), may be taken to be approximately proportional, within each species, to the abundance-level of the adults.* They can not, however, give any information as to the abundance of the different species relative to each other, since we do not know their relative catchability. As measures of abundance, the daily catches suffer from the effects of the unknown factors responsible for the significant residual variability remaining, at least in the case of the moths, after correction for the temperature effect (Section V(*b*)); averaging over suitable short periods may be expected to minimize this defect. In Figure 8 the catch data, treated in this way, have been plotted against time over the whole period of the investigation.

Estimated values have been used to fill certain gaps in the record. The most serious gap is that between late May and late October 1943, when trapping was temporarily discontinued: nothing can be done to fill this. Prior to the installation of the cubical trap in April 1942, we have stack-trapping data, for the moths only, extending back to mid-February (Section IV(*b*)). These were utilized by calculating their "cubical trap equivalent" on the basis of the data for the week in April during which both methods of trapping were employed. On several individual dates, and for a more extended period in October 1942, the two species of moth were not distinguished in recording the catch. In these instances, estimates for each species were made by distributing the total catch in the ratio of the catches for the two species over a period of a fortnight straddling the dates concerned.

The complete series of catch data, observed and estimated, was corrected for the temperature–activity effect to the appropriate standard temperature. In so doing, the assumption was made that the same correction factor could validly be applied to the female moths as to the males; the issue is not an important one,

*To the extent that the activity–temperature regression may have been contaminated by a short-term effect of temperature on adult emergence, the abundance will have been underestimated at the higher temperatures relative to the lower.

since females never constituted more than about 11 per cent. of the total. For the period prior to April 21, 1942, the flight period temperatures are estimates derived from the thermograph trace of the Brisbane climatological station by means of a regression between store and screen temperature over the period April 21 to May 6, 1942.

The corrected catch data were then grouped in periods of usually a fortnight and the mean daily values calculated. In Figure 8 these have been plotted in the transformation log $(n+1)$. Portions of the plot based on estimated values are shown in thinner line.

Rigorous analysis of the resulting curves of adult abundance is not possible. However, certain relations are quite evident and at other points plausible interpretations can be offered.

There is an astonishing correspondence, even in minor details, between the curves for the two species of moth. No consistent trend in the ratio of their catches is apparent over the 22-month period and we must conclude that they reacted in almost identical fashion to the various factors influencing their abundance. Numbers were very low in the winters of both years. In 1942 they rose sharply from a minimum in July, reaching a peak in October. In mid-November they declined to about half the October level, rising again to a higher peak in mid-December. In the autumn of 1942 the abundance had fluctuated, but it experienced little overall decline until May.* We cannot tell whether prior to February it had attained a level comparable with the peaks of October and December 1942. On the other hand, in the autumn of 1943 it was falling as early as February at about the same rate as in the May–July period of 1942; it had already been falling at a somewhat reduced rate from mid-December, and by mid-May, i.e. 2 months earlier than the minimum of 1942, it reached zero. It seems likely that mortality factors were operating more strongly in the autumn of 1943 than in 1942.

The very limited data for the spring and early summer of 1943 are sufficient to establish that the abundance of the moths was very much lower than in the corresponding period of 1942. If we compare the levels for the same date in early December, the 1943 figure is only about 1/30 of the 1942. On the other hand, if we regard the early November figure for 1943 as corresponding to the late October figure of 1942 and the early December figure to the interpeak minimum of November 1942, we arrive at a value of about 1/12 for the ratio of the 1943 to the 1942 level. We cannot tell to what level the spring maximum attained in 1943, but if we assume that the rise commenced in July and the curve had a similar shape to that of 1942, the maximum can hardly have been greater than 1·5 of that in 1942. Apparently mortality factors were operating still more strongly in the spring of 1943 than in the previous autumn.

Turning now to the curve of adult abundance of *Apanteles*, we see that it corresponds only very broadly with the moth curves. There is the same low level in the two winters, but the parasite curve both rises and falls more slowly than the moth, with the result that the ratio of abundance of parasites to moths (although

*The small peak in early April may have been influenced by disturbances in the store. At this time some restacking was carried out, affecting stack 5R amongst others.

we can conclude nothing as to its absolute value) rises during the later part of the season and falls during the earlier. In 1943 and possibly also in 1942 the parasite peak occurred later than the moth peak; there is no definite evidence of a parasite minimum corresponding to the interpeak moth minimum of November 1942. The parasite peak seems to have occurred appreciably earlier in 1943 than in 1942. Certainly the winter minimum was virtually attained 2 months earlier in 1943, the parasite agreeing in this respect with the moths. Again, in the spring and early summer of 1943 the parasite abundance was much lower than in the corresponding period of 1942. The data for the parasite suggest, as for the moths, a steady increase in the impact of mortality factors from about the beginning of 1943 onwards.

The winter troughs in moth and parasite abundance are probably a direct temperature effect. At the top of Figure 8 is shown the progression of the daily maximum thermograph temperature, averaged, for the most part, over the same periods as the moth and parasite data. The portions of this curve shown in thinner line are based on estimates of the store maximum obtained from the screen maximum (cf. Fig. 4) and are subject to a maximum error of about $\pm 3°$F. It has been concluded in Section II(*c*)(i)(1) that the lowest temperature ever attained in the store was about 50°F. This is very far from being lethal to any stage of either species of moth and is only a little below their threshold of development (Griswold 1944; Titschack 1922; Cheema 1956). On the other hand, the fortnightly mean maximum never fell below 67°F (Fig. 8), while Figure 4 shows that the monthly mean temperature [(maximum + minimum)/2] never fell below 63·5°F. Thus we must conclude that the moths, and probably also the parasite, were able to continue their development throughout the winter, although at a much reduced rate. If the retardation affected the larva and pupa more than the adult, i.e. if the emergence rate of adults was reduced more than their death rate, the adult abundance would fall.

The numbers of adult moths began to rise again in 1942 while the temperature was still at its lowest point. We may suppose that this was due to two factors: the accumulation of mature pupae only awaiting the stimulus of an above-average daily maximum temperature for emergence, and the fact that by then most of the old adults would have been replaced by young ones.

Over the periods of higher adult abundance the effects of temperature are less clear. From the work of Rawle (1951), Cheema (1956), and others, it would seem that even a few hours at 93°F must result in appreciable mortality of all stages of both species of moth. We have seen in Section II(*c*)(i)(1) that the highest temperature experienced at the level of the top of the stacks must have been *c.* 100°F, and that a difference of *c.* 7°F can exist between temperatures at that level and at the level of the thermograph. This figure of 7°F is neither a maximum nor a mean, but if we use it to give some indication of what thermograph temperature might correspond to a figure of 93°F at the top of the stacks, we get the value 86°F. The numerals associated with certain of the points on the temperature curve in Figure 8 represent the number of days, within the respective periods, on which the maximum temperature reached or exceeded this value. It is evident that in each of the three warmer periods there were a number of days on which lethal tempera-

tures could have prevailed for a time in at least the uppermost tier of the stacks. However, the effect of such mortality on adult abundance over the store as a whole (which is what the cubical trap measures) is quite problematical. Not only have we no information as to how large or small a proportion of the total adult population was exposed to the lethal temperatures, but it is clear also that lethal temperatures at the top of the stacks would have their counterpart in more favourable (warmer) conditions in the lower parts (see Table 1) and that these could be expected to result in a compensating increase in emergence.

In actual fact, the moth record shows on the whole a positive correlation with temperature during the period of high temperatures in March to early May of 1942 and a rather striking negative correlation during the corresponding period October to December. However, the latter must be interpreted with caution. The decline in adult abundance between October and mid-November is between half and two-thirds of the October value—over a period when only two days are likely to have experienced lethal temperatures at the top of the stacks. We have assumed that the steep spring rise in adult moth numbers was due to the progressive transformation of overwintering larvae, and it is probable that the main cause of the November trough was simply that this process had been largely completed and mortality of senile adults was once more in excess of new emergences—including lagging members of the winter brood plus the vanguard of the new generation. The December peak would then indicate the ascendency of the new generation. On this interpretation the double peak in the closing months of 1942 would be evidence of the occurrence of two widely overlapping generations, with perhaps some minor added temperature effects. The period from about the end of August, when the spring generation of adults was well established, to the end of November appears to be adequate for the development of a generation at the temperatures prevailing (an overall mean of 72·7°F, see Fig. 4), even on a food of somewhat inferior quality. Thus at constant temperatures between 70 and 80°F various authors (Titschack 1925; Billings 1936) have recorded for *Tineola* developmental periods of 2–3 months on contaminated or supplemented wool; Cheema (1956) records about 7 weeks for *Tinea* on yeast-impregnated woollen fabric at 77°F.

The single seasonal peak in *Apanteles* suggests that in this case we are dealing with a succession of several completely overlapping generations, for Fallis (1942) found that at 75°F the species completes its life cycle in about 6 weeks and at 80°F in 26–28 days.

We have already noted that the course of the curves after the peak of December 1942 differs in important respects from their course during the autumn of 1942 and we have concluded that mortality factors must have borne more heavily on the populations during the later period. The early part of the decline, in December 1942 and January 1943, might reasonably be ascribed to a repetition of the generation effect, assisted, perhaps, by the occurrence of a number of days with lethal temperatures at the top of the stacks. However, on this assumption another peak would have been expected to occur not later than March 1943. Under the temperatures prevailing (overall mean of 77·4°F for December–February, Fig. 4), this interval should have been ample for the completion of a third generation,

and the March temperatures, while very favourable for development (mean 76·7°F), did not reach lethal levels, as they did in 1942. Actually the adult abundance fell steadily and progressively throughout this period. A slight check of very doubtful significance occurred in the rate of fall early in March, but the abundance at that time was only a quarter of its level in March 1942. Thus the question arises as to the nature of the mortality factors involved.

The food supply of the larvae could not have been deficient in quantity and it is also unlikely that any serious reduction had occurred in the availability of faeces-, urine-, and burr-contaminated wool, which constitutes the preferred food (Section III). It is possible that predacious mites had got a hold on the moth populations, or that the larvae were suffering from a disease. We found no evidence of the former. Although we have found numbers of dead larvae in opened dumps, especially of older wool, the cause of death was not established and we cannot be sure that the mortality was higher in 1943 than in 1942. The ratios of the abundance estimates of *Apanteles* to those of the moths (again these ratios have no absolute significance) were practically the same during the periods of decline in the two years. The parasite did not prevent the larvae of the late autumn and winter of 1942 from giving rise to an enormous adult moth population in the spring, so that it does not seem possible to ascribe the failure of a moth peak in the autumn of 1943 to this agency, even if the temperatures prevailing over the relevant period did, as is likely, allow of a greater number of *Apanteles* generations than in 1942. On the other hand, the fall in the abundance of *Apanteles*, which, like that of the moths, occurred much earlier in 1943 than 1942, would be consistent with mortality of the host larvae from some other cause; but it could also have been determined in other ways, as will be indicated below. Although quantitative data are not available for the second clothes moth parasite, *Chremylus rubiginosus* (see Section II(*d*)), there was no evidence of any striking increase in its abundance and no suggestion that it might be responsible for the rising mortality.

A quite unusual circumstance provides the clue to what is very probably the true explanation not only of the increased mortality in the autumn of 1943, but also of the catastrophic reduction of the adult populations the following spring. The female percentage in the catch of *Tineola* and *Tinea* combined remained constant during 1942, but rose steadily thereafter until November 1943 (Table 9). The difference between the value of 0·28 for the mean female percentage in 1942 and the 11·38 per cent. in November 1943 is equivalent to a 40-fold increase in the relative abundance of females. It would seem that such a change could only be brought about by a mortality factor operating selectively against the adult male, and the only basis for such discrimination would seem to be the relative flight activity of the two sexes. In other words, some agent was eliminating moths in flight. Two such agents are known to us: the cubical trap and the spider *Uloborus geniculatus* (Section II(*d*)).

In March 1944 the webs of *Uloborus* were straddling all the gaps between the vertical faces of dumps adjacent to the passages at an average density of about six per foot (Section II(*d*)). A considerable time would have been required to bring them to this level of abundance: they must have been numerous already at the

beginning of 1943, although no figures are available. Evidence has been cited to show that the spiders did catch and feed upon the moths and that no other source of food could have maintained so large a spider population. In comparison with the combined effect of the spiders throughout the store, the 2-ft-cube trap could have had no appreciable effect on moth abundance. Thus the spiders must be credited with bringing about the 40-fold increase in the percentage of adult females, by selective removal of males. To achieve this, they would need to reduce the total catch to 1/40 of what it would otherwise have been and the population of adult males to about 1/45, assuming that they caught no females. The latter assumption is, of course, very unlikely to be true. The spiders may be expected to have caught females at least in the proportion of the cubical trap and probably in a considerably

TABLE 9

SEX RATIO IN SAMPLES OF THE CATCH OF TINEOLA AND TINEA COMBINED, BY MONTHS AND GROUPS OF MONTHS

Period	No. of Males	No. of Females	Females (%)
April–June 1942	2129	8	0·37
July–September 1942	4415	8	0·18
October–December 1942	4286	14	0·33
January 1943	3067	23	0·74*
February 1943	1358	25	1·81*
March–May 1943	644	20	3·01
October 1943	181	15	7·65*
November 1943	257	33	11·38
December 1943	120	6	4·76*

*Significantly different from the immediately preceding period.

greater proportion, since the webs of the smaller specimens bridged the narrowest gaps between the dumps, where females were likely to become entangled in them perhaps even without taking flight. Any removal of females in this way would diminish still further the fraction to which the adult population would need to be reduced in order that, by November 1943, females should constitute 11·38 per cent. of the catch. The fall in the female percentage between November and December 1943 is unexplained.

The data of Table 9 have been included in Figure 8 in the transformation log [(No. of females/1000)+1]. They have also been used, without transformation, to construct a smoothed curve of female percentage against time. From this, estimates of the female percentage were read off for the dates, from October 17, 1942, onwards, corresponding to the individual points on the abundance curves. The date October 17, 1942, has been taken as that from which the female percentage can be said to have started rising above the 1942 norm of 0·28. The estimates were used to calculate, for each date, the theoretical catch of each moth species that would have been obtained had the normal sex ratio (and hence percentage of

females in the catch) been maintained, assuming removal of males only. This value is given by the expression

$$(\text{actual catch} \times \text{estimated female percentage})/0{\cdot}28.$$

The calculated catches for *Tineola* have been plotted in Figure 8 in the transformation log $(n+1)$. For the reasons already given, they represent a conservative estimate of the abundance level assuming no spider predation. They would need to be increased in the proportion (unknown) to which the predator had reduced also the female abundance.

It may be seen that the calculated catch departs increasingly from the actual during the period of decline in the late summer and autumn of 1943, and in the following spring reaches a level comparable with that for December 1942. The steep fall in May 1943 is clearly due to the fact that the actual catch reached zero at that date: if the trend of the curve is allowed to continue roughly parallel to the curve of actual catch, it reaches zero at about the end of June, i.e. at much the same time as the minimum of 1942. However, the calculated curve still lacks the expected autumn peak in 1943.

The wholesale removal of males by the spider is likely to have had unfavourable effects on reproduction. A reduction of male abundance to 1/45 in November 1943 would convert the normal sex ratio of 2 males to 1 female suggested for *Tineola* in Section V(*b*) into one of more than 20 females to 1 male. Unless copulation normally precedes flight, it seems inevitable in such circumstances that some females would remain unfertilized and leave no progeny. Others would suffer a delay before copulation, and Titschack (1926*b*) has shown that this results in lowered egg production. The same consequences may be expected, in a lesser degree, from the less drastic disturbance of the sex ratio already apparent in the autumn of 1943. Thus, in spite of the fact that the spider predation was directed so largely against the males, the capacity of the population to increase must have been gradually undermined. This may well be the reason for the failure of the autumn peak in 1943 even in the calculated curve; increasing overlap of generations may also have contributed. We have already noted (Section V(*b*)) that delayed copulation might result in an increase in female activity relative to male. There is no evidence for this, but in so far as it may have occurred, the calculated catch will have been overestimated.

A similar calculated curve could be derived for *Tinea* and would show a similar relation to the actual curve. In this species, however, we have to assume a normal sex ratio of 4 females to 1 male (Section V(*b*)), which would be increased by November 1943 to about 180 : 1. A correspondingly greater interference with reproduction might be expected in comparison with *Tineola*. However, there is no evidence of such a difference in the relations of the curves of actual catch of the two species by the spring of 1943.

We may suppose that the spider would also have been catching adult *Apanteles*, although we have no means of judging to what extent. This may well have accounted in large part for the early decline of *Apanteles* abundance in the autumn of 1943 and its low level in the following spring, for there is no suggestion in Figure 8 that the parasite was being limited by food shortage.

VIII. Discussion

We know of no investigation similar in scope to ours and carried out on a very large undisturbed population of clothes moths. Herrick (1933) made a few observations on a much smaller population of *Tineola bisselliella* infesting fleeces that had been stored for two years in a warehouse. Collins and Glasgow (1946) report investigations (made subsequent to ours) on a population of comparable size in a multi-storeyed warehouse in the United States, but they were concerned solely with control measures. The work of Richards and Waloff (1946) and Waloff and Richards (1946) on *Ephestia elutella* Hübn. in bulk grain had objectives similar to ours and affords some interesting comparisons. Our technique of population sampling with tanglefoot traps has apparently not been used before in a study of this kind. Collins and Glasgow (1946) employed light-suction traps. Doner and Thomssen (1943) refer to the use by "Wilson" of fly-papers as a control measure, but give no reference.

The conditions in C store were extremely favourable to clothes moths. As food supply there was an unlimited quantity of suitably contaminated, fine Merino wool. The significance of contamination has already been mentioned; Meeuse (1951) states that fine fibres are more readily attacked by young larvae than coarser ones. The temperature fluctuated within a narrow range in the free air spaces and within a still narrower range in the surface wool of the bales. In the former situation it was never low enough to arrest the development of any stage, except briefly, and only a small proportion of the population was ever exposed to a temperature high enough to be lethal. It is probable that at a depth of 2 in. inside the bales neither lethal effects nor arrest of development ever occurred. The overall annual mean temperature, which would be the same in the wool as in the free air spaces, was in the vicinity of 72°F (22°C). There seems to be fairly general agreement that the optimal temperature for rearing both species of moths is in the range 24–26°C (Titschack 1922; Notini 1939; Meeuse 1951; Cheema 1956). Humidity data for the store are not satisfactory, but it seems likely that the overall annual mean lay in the range 50–70 per cent. Although clothes moths appear to be relatively insensitive to humidity differences, Meeuse (1951) suggests 60 per cent. as the optimum for laboratory rearing of *Tineola bisselliella*; the figure may be higher (Cheema 1956) for *Tinea pellionella*.

Under the conditions described, it might be supposed that the moths would increase in numbers until very serious damage was being done to the wool. Some justification for such a fear was afforded by the tremendous increase in the population that must have taken place between the filling of the store in late 1940 and the commencement of the present observations a year later. In actual fact, as we have seen, the population reached a maximum in the summer of 1942 and then declined rapidly, so that in the summer of 1943 it was only a small fraction of the maximum. We have definite evidence of only three natural enemies of the moths in the store: the parasites *Apanteles carpatus* and *Chremylus rubiginosus* and the spider *Uloborus geniculatus*. It has already been concluded that the parasites could not have been responsible for the decline. The more abundant species, *Apanteles carpatus*, was handicapped in at least two respects. Although the female may lay

several eggs in a single host larva, only one matures (Fallis 1942); and there is every reason to believe that the adult parasites were preyed upon heavily by the spiders. Further, Fallis found that the adults fed on diluted honey in the laboratory, although he does not state whether feeding was necessary for normal fecundity: there would seem to have been no source of similar food in the store. Thus the only likely controlling factor is predation by *Uloborus*, and we have seen that both the enormous numbers of spiders present at the end of the relevant period and the fact that the increased mortality fell almost entirely on the males (which fly) lead us very strongly to the conclusion that this was indeed the factor responsible.

To our knowledge this is the first occasion on which it has been claimed that a spider has been responsible for limiting ("governing", in the terminology of Nicholson (1954)) the population of an insect. There is, however, evidence that spiders may be important enemies of moths under the special conditions prevailing in warehouses and similar storage places. Thus Titschack (1922) found a species *Tegenaria domestica* (L.) preying upon his mass cultures of *Tineola*. The same species became common in the second year of Richards and Waloff's study (1946) of *Ephestia elutella* in a London grain store. These authors estimated a rate of predation of two moths per spider per fortnight. However, this estimate may be far too low, for it was based on dead moths observed in the webs, while Titschack (1922) states that *Tegenaria* completely consumes its prey, leaving no residue. Even if this is not strictly true, it seems likely that Richards and Waloff's spiders may have consumed far more moths than were noted in the webs. For a particular date Richards and Waloff (1946) give the sex distribution of the moths found in 101 webs as 115 males to 16 females, the sex ratio in the moth population being 9 males to 2 females at the time. Thus we have here direct confirmation of the assumption that spiders would catch more of the more active sex—according to Waloff and Richards (1946) males predominate in flights of *E. elutella* but a substantial amount of female flight also occurs.

Several circumstances seem likely to have favoured *Uloborus* in acquiring the role of limiting factor on the clothes moth populations in our investigations. In the first place, the food supply could not directly limit the moths for many years, while the parasite *Apanteles carpatus* was severely handicapped and was itself a prey of the spider. Secondly, the spider was confronted with an abundant food supply (chiefly the moths), of a size probably suited to most of its developmental stages; it was probably strongly favoured by the physical conditions of temperature, humidity, and light intensity, and by the construction of the stacks, which seemed to provide almost ideal supports for the webs of spiders of all ages—supports, moreover, which constituted at the same time both the food and the refuges of the moths. Thirdly, larger predators of the spider may not have been able to gain entrance to the store, or else they, along with spider parasites, may not yet have had time to influence appreciably the rapidly increasing spider population.

Trapping in C store ceased in December 1943 and the store was not visited after March 1944. It is understood that shortly afterwards all wool was removed. Thus we can only surmise what the future of the little animal community would

have been if conditions had remained undisturbed. It seems clear that the moth populations would have fallen almost to extinction. The spiders would probably have turned to cannibalism until their webs were more widely spaced, after which their numbers would no doubt have crashed. In time, some sort of equilibrium would have been established, probably at a rather low level of both predator and prey, provided that no new element was introduced. Although the community in the store was a very simple one, the environment was very specialized and lacking in diversity, and it may well be that few additional species would be capable of living there in significant numbers. However, the establishment of an efficient parasite or predator of *Uloborus*, for example, might well deprive that species of its importance as a control of the moths and lead to the restoration of high moth densities.

IX. Acknowledgments

The work here described could not have been undertaken without the cooperation of the Queensland State Wool Committee and its chairman, Mr. A. Campbell. At some inconvenience, the Committee guaranteed us the use of C store and its wool, free from major disturbance, for a period of two years.

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X. References

Billings, S. C. (1936).—Notes on clothes moth breeding. *J. Econ. Ent.* **29**: 1014–16.

Cheema, P. S. (1956).—Studies on the bionomics of the case-bearing clothes moth, *Tinea pellionella* (L.). *Bull. Ent. Res.* **47**: 167–82.

Collins, D. L., and Glasgow, R. D. (1946).—DDT thermal aerosol fogs to control clothes moths in a wool storage warehouse. *J. Econ. Ent.* **39**: 241–5.

Common, I. F. B. (1954).—A study of the ecology of the adult bogong moth, *Agrotis infusa* (Boisd.) (Lepidoptera: Noctuidae), with special reference to its behaviour during migration and aestivation. *Aust. J. Zool.* **2**: 223–63.

Doner, M. H., and Thomssen, E. G. (1943).—Clothes moths and their practical control. *Soap, N.Y.* **19**(10): 102–5.

Fallis, A. M. (1942).—The life cycle of *Apanteles carpatus* (Say) (Hymenoptera : Braconidae), a parasite of the webbing clothes moth, *Tineola bisselliella* Hum. *Canad. J. Res.* D**20**: 13–19.

Griswold, G. H. (1931).—On the length of the adult life in the webbing clothes moth, *Tineola bisselliella* Hum. *Ann. Ent. Soc. Amer.* **24**: 761–4.

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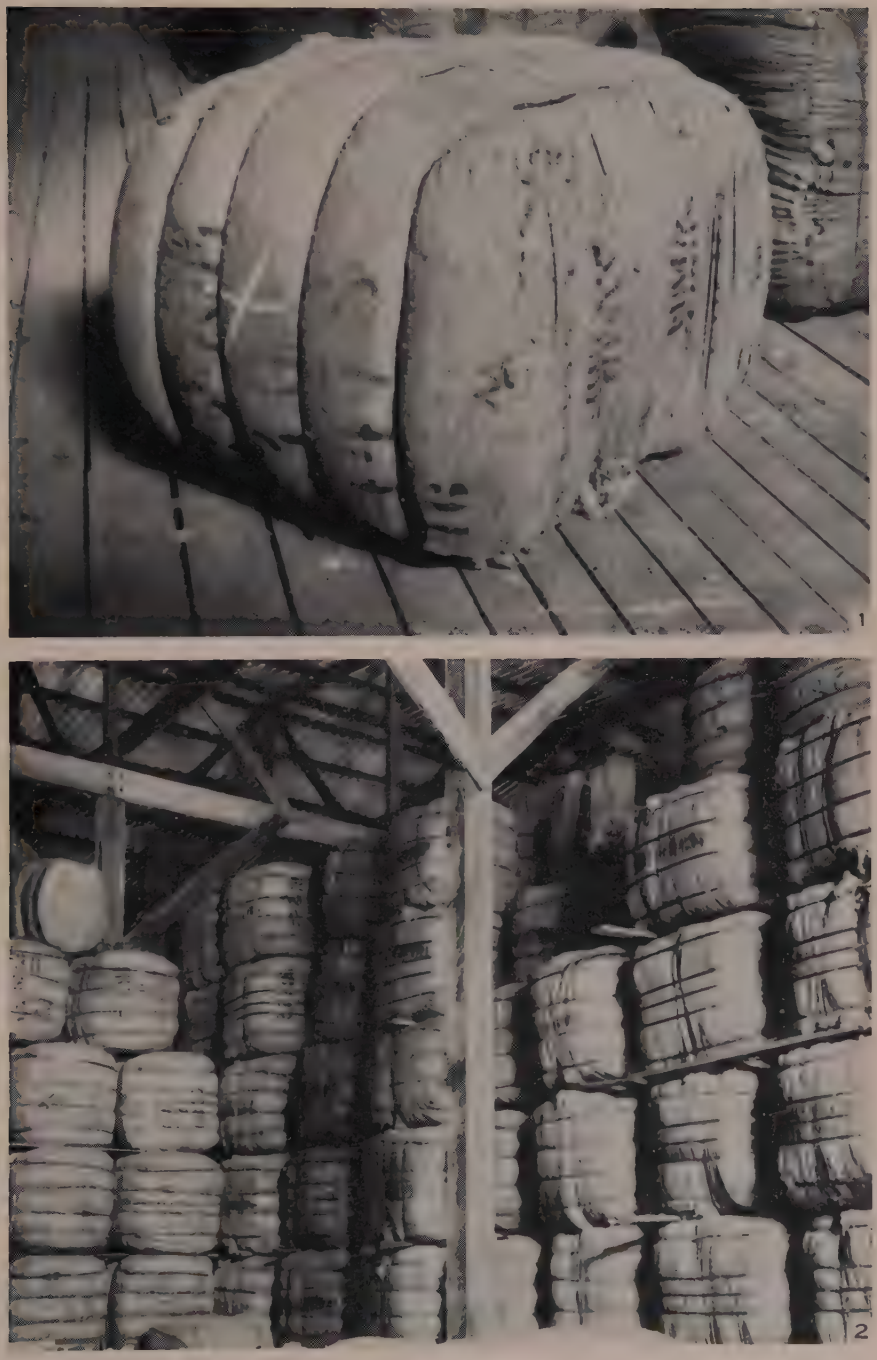

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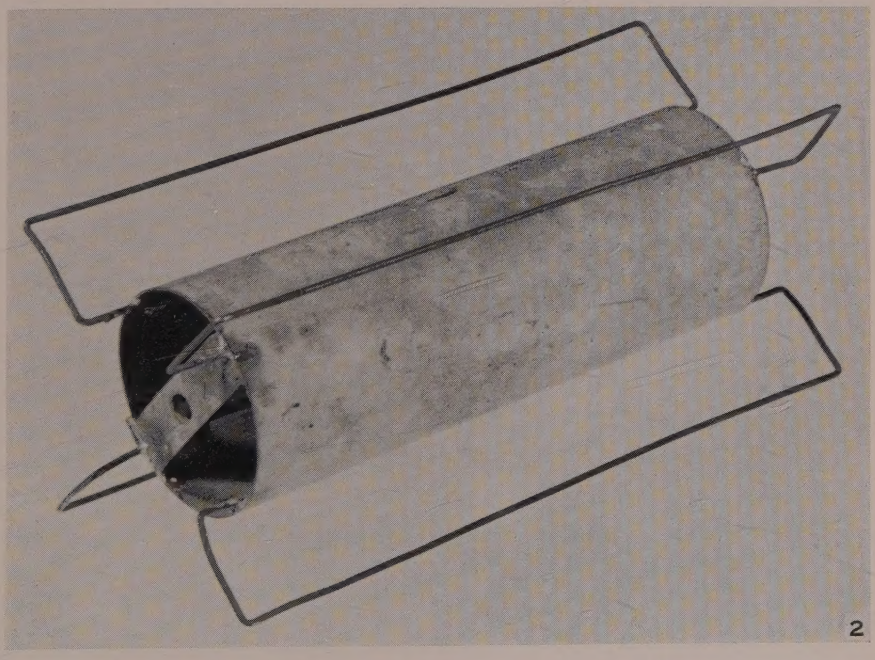

GRISWOLD, G. H. (1944).—Studies on the biology of the webbing clothes moth (*Tineola bisselliella* Hum.) Mem. Cornell Agric. Exp. Sta. No. 262. 59 pp.

HERRICK, G. W. (1933).—An unusual invasion of the clothes moth, *Tineola bisselliella* (Lepid.: Tineidae). *Ent. News* **44**: 99–101.

MEEUSE, A. D. J. (1951).—Mothproofing from the entomological point of view. Meded. Vezelinst. T.N.O. No. 109. 45 pp.

NICHOLSON, A. J. (1954).—An outline of the dynamics of animal populations. *Aust. J. Zool.* **2**: 9–65.

NOTINI, G. (1939).—Klädesmalen. Medd. Växtskyddsanst. Stockh. No. 28. 32 pp.

RAWLE, S. G. (1951).—The effects of high temperature on the common clothes moth, *Tineola bisselliella* (Humm.). *Bull. Ent. Res.* **42**: 29–40.

RICHARDS, O. W., and WALOFF, N. (1946).—The study of a population of *Ephestia elutella* Hübner (Lep., Phycitidae) living on bulk grain. *Trans. R. Ent. Soc. Lond.* **97**: 253–98.

TITSCHACK, E. (1922).—Beiträge zu einer Monographie der Kleidermotte, *Tineola biselliella*. *Z. tech. Biol.* **10**: 1–168.

TITSCHACK, E. (1925).—Untersuchungen über den Temperatureinfluss auf die Kleidermotte (*Tineola biselliella* Hum.). *Z. wiss. Zool.* **124**: 213–51.

TITSCHACK, E. (1926*a*).—Ueber die imaginale Lebensdauer der Kleidermotte, *Tineola biselliella* Hum. *Verh. naturh. Ver. preuss. Rheinl.* **82**: 330–48.

TITSCHACK, E. (1926*b*).—Untersuchungen über das Wachstum, den Nahrungsverbrauch und die Eierzeugung. II. *Tineola biselliella* Hum. Gleichzeitig ein Beitrag zur Klärung der Insektenhäutung. *Z. wiss. Zool.* **128**: 509–69.

TITSCHACK, E. (1936).—Experimentelle Untersuchungen über den Einfluss der Massenzucht auf das Einzeltier. *Z. angew. Ent.* **23**: 1–64.

WALOFF, N., and RICHARDS, O. W. (1946).—Observations on the behaviour of *Ephestia elutella* Hübner (Lep., Phycitidae) breeding on bulk grain. *Trans. R. Ent. Soc. Lond.* **97**: 299–335.

WILLIAMS, C. B. (1940).—An analysis of four years' captures of insects in a light trap. Part II. The effect of weather conditions on insect activity; and the estimation and forecasting of changes in the insect population. *Trans. R. Ent. Soc. Lond.* **90**: 227–306.

EXPLANATION OF PLATES 1–3

PLATE 1

Fig. 1.—A typical war-time "double dump". Face I front left, III front right, II top. Note wool protruding through breaches in the pack.

Fig. 2.—Interior of C store showing parts of two stacks. The ridge of the roof runs from left to right at the top of the picture; the main passage (not visible) is beneath it. In the foreground part of a stack has been dismantled; the undismantled portion is represented by the dumps on the left, exposing face I to the camera. The dumps to the centre and right, exposing face II to the camera, belong to a second stack, separated from the first by an alley.

PLATE 2

Figs. 1 and 2.—Two views of a stack face, showing webs of the spider *Uloborus geniculatus* straddling the vertical gaps.

PLATE 3

Fig. 1.—Looking east in alley 4R, with the cubical trap in position. Stack 4R to left, 5R to right. Bridging the alley behind the trap is the board to which the thermohygrograph (out of view behind the trap) was attached.

Fig. 2.—Cylindrical trap, 6 in. long by 2 in. in diameter, as finally adopted for trapping within stacks. Note protective wires and cross-piece with hole to take string.